UNIVERSIDAD COMPLUTENSE DE MADRID

Facultad de Ciencias Biológicas
Departamento de Zoología y Antropología Física

TESIS DOCTORAL

Contribution to the knowledge of cyclorhagid mud dragons (Kinorhyncha Cyclorhagida: biogeograph, taxonomy, morphology.

Contribución al conocimiento de los kinorrincos ciclorrágidos (Kinorhyncha Cyclorhagida) : biogrografía, taxonomía, morfología

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

María Herranz Matesanz

Director

Fernando Pardos Martínez

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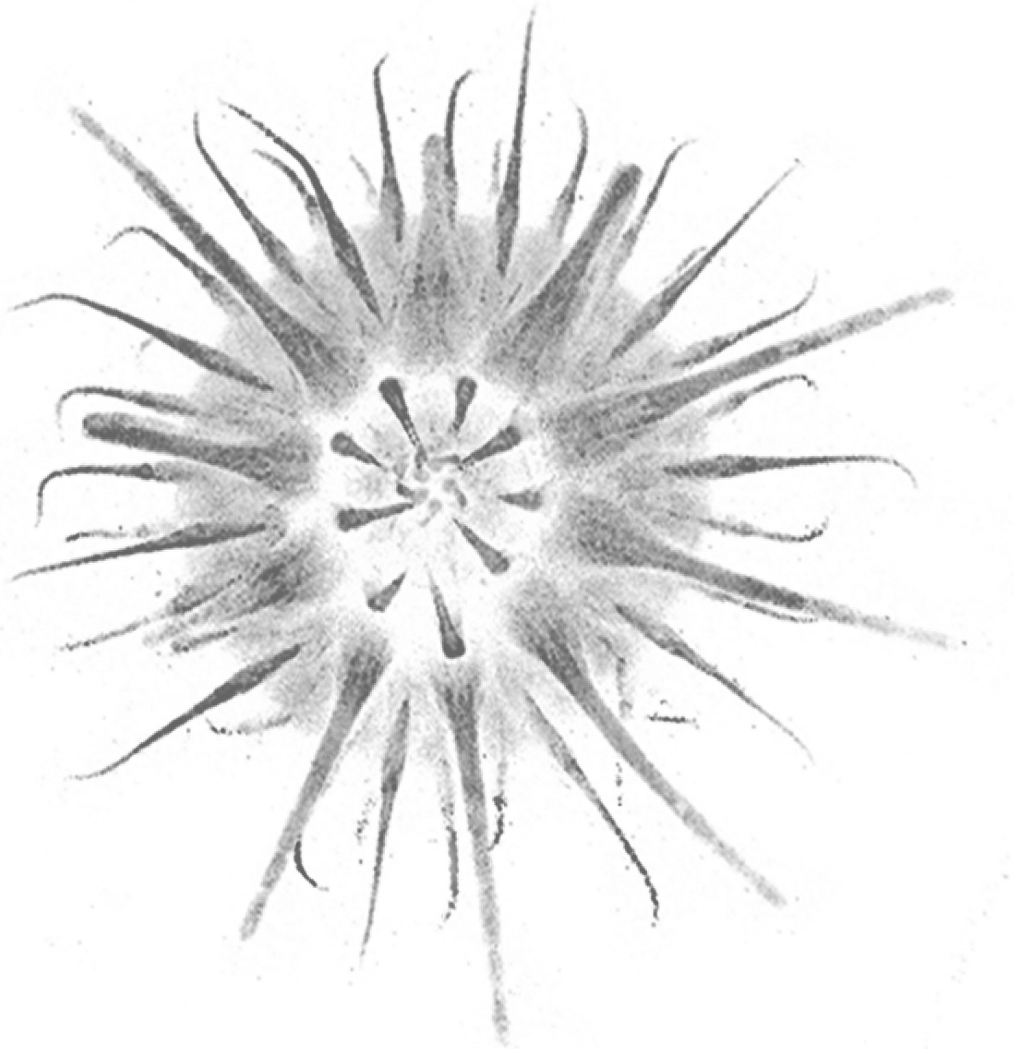
Tesis Doctoral 2014



Departamento de Zoología y Antropología Física

Facultad de Ciencias Biológicas
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FERNANDO PARDOS MARTÍNEZ, PROFESOR TITULAR DEL DEPARTAMENTO DE ZOOLOGÍA Y ANTROPOLOGÍA FÍSICA DE LA FACULTAD DE CIENCIAS BIOLÓGICAS DE LA UNIVERSIDAD COMPLUTENSE DE MADRID, CERTIFICA:

Que la presente memoria titulada "Contribución al conocimiento de los kinorrincos ciclorrágidos (Kinorhyncha, Cyclorhagida): biogeografía, taxonomía, morfología" presentada por Dña. María Herranz Matesanz para optar al Título de Doctora en Biología, ha sido realizada en el Departamento de Zoología y Antropología Física de la Facultad de CC. Biológicas de la Universidad Complutense de Madrid bajo mi dirección. Y considerando que representa trabajo de Tesis, autorizo su presentación a la Junta de Facultad.

Y para que así conste, firmo el presente en Madrid, Marzo 2014

La Doctoranda *V°B° del director*

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A mis padres y mi hermana.
A mi familia

To see a World in a grain of sand,
And a Heaven in a wild flower,
Hold Infinity in the palm of your hand,
And Eternity in an hour...

William Blake
Auguries of Innocence

Contents

Abstract

Background

Kinorhyncha is a phylum of microscopic free-living marine invertebrates exclusively meiobenthic, ranging in size from 0.1 to 1mm. They are one of the several phyla included in the meiofauna, considered the group of benthic animals that have intermediate sizes between macro and microfauna. Kinorhynchs are widely distributed and have been reported from polar to tropical regions and from the intertidal to abyssal depths (max. 7800 m), mostly found in the upper 2-3 cm centimeters of the muddy sediment, influenced by the oxygen availability and food supply. However, they can occupy other habitats such as algae, stones, or the surface of different metazoans (bivalves, polychaetes, bryozoans, sponges, etc.). Kinorhynchs seem to graze on detritus, algae, bacteria and diatoms but specific data about their feeding is still scarce. They have been reported as food source of bivalve molluscs, fish of the family gobiidae, polychaetes, and shrimps and it is most likely that they can also appear in the digestive tracts of other deposit-feeding animals, however no direct evidence has yet been reported.

The body of kinorhynchs is divided into three regions: head, neck, and trunk. The head is composed of an eversible introvert and a protrusible terminal mouth cone. Both structures can be retracted into the trunk and are involved in the feeding and motion of the animal. The mouth cone carries four rings of cuticular appendages usually arranged following a pentaradial symmetry. The introvert bears several cuticular sensory and locomotory appendages, named scalids, arranged radially and staggered in up to seven concentric rings. The neck is composed of a set of small and cuticularized plates called placids that generally articulate with the first trunk segment acting as a closing apparatus when the head is retracted into the trunk. The trunk in adult kinorhynchs and juveniles of the last stage is composed of 11 articulated segments which connect each other through a soft cuticular intersegmentary joint allowing the animal to be highly flexible. Each trunk segment can be either ring-like or subdivided longitudinally in up to four plates, dorsal (tergal) and ventral (sternal), that articulate with each other. The trunk is equipped with many different cuticular structures distributed segmentally showing an extraordinary morphological diversity among species and genera being very important as diagnostic characters. Indeed, the type and arrangement of these cuticular specializations is normally unique at species level. Segmentation in kinorhynchs is evident externally, however internally not all the organ systems mirror the same pattern. Muscles, nerves and glands show an apparent segmental arrangement whereas gut, reproductive and excretory organs are non-segmental. Moreover, respiratory and circulatory systems are absent.

Phylogenetically kinorhynchs are positioned within the Ecdysozoa, one of the two protostome monophyletic superclades including arthropods, tardigrades, onychophorans, nematodes, nematomorphs, loriciferans and priapulids. Within Ecdysozoa, kinorhynchs are allied to nematomorphs, nematodes, priapulids and loriciferans in the clade Cycloneuralia sharing a collar-shaped brain surrounding the pharynx. Within Cycloneuralia priapulids, kinorhynchs and loriciferans form the Scalidophora on the basis of a shared introvert with scalids and the presence of two rings of retractor muscles in the introvert. Most of the phylogenetic studies place kinorhynchs together with priapulids as sister groups while the position of loriciferans remain less clear. The phylogeny within Kinorhyncha is still far to be resolved, however recent studies are starting to include molecular data.

To date the phylum Kinorhyncha comprises around 210 species (based on descriptions of adults) nested

in 23 genera. During the last 10 years the number of species and genera described has significantly increased meaning that a great part of the biodiversity of the phylum is still unknown. The present Thesis is focused in the study of one of the two extant orders of kinorhynchs, the cyclorhagids, which are the most widely distributed and diverse order accommodating 17 genera and 130 species so far. Cyclorhagid kinorhynchs are characterized by the presence of a neck composed of 14-16 placids, a ring-shaped first trunk segment, which is round to oval in cross section, and cuticular spines along the trunk.

State of the art

Nowadays, less than ten people form the international community of active researchers dedicated to the study of kinorhynchs. Current investigations on the phylum are focused both in the study of unexplored areas to describe new taxa and in the study of morphology trough modern techniques such as the confocal laser microscopy studies included in the present thesis. Due to their small size, cyclorhagid kinorhynchs are particularly difficult to work with and therefore the knowledge of their internal organ systems is still very limited and merely reduced to scattered transmission electron microscopy studies.

With the course of the years and the improvement of the microscopic techniques, the taxonomic important characters and terminology for cyclorhagid kinorhynchs have been modified and new terms have been included making the descriptions more accurate and specific. On the other hand new phylogenetical studies are starting to include molecular data. However, these studies need to be improved to get a better resolution in order to resolve with more detail the relationships among the phyla. Unfortunately, embryologic and developmental studies are not being successful due to the difficulties presented in the culturing of kinorhynchs.

The current information about the concrete geographical distribution of cyclorhagid kinorhynchs is extremely fragmented and merely a reflection of the activities of the few experts that have studied the group through time. The Iberian Peninsula was almost an unexplored area until the late 1990s when our research group initiated a series of pioneer projects funded by the Spanish government. This thesis began in the framework of these investigations including also studies of additional areas.

Main objective

Within the previous context it seems clear that the studies of cyclorhagid kinorhynchs are still in an early stage. There is a substantial lack of knowledge in many areas of study such as taxonomy, biogeography, ecology, morphology, whereas others are just starting to be explored, for instance the molecular phylogeny and the developmental biology. According to this situation, the general objective of this Thesis is to contribute to integrate three different approaches (biogeographical, taxonomical and morphological) to increase the current knowledge of the cyclorhagid kinorhynchs with a broad perspective. In order to accomplish it, I carried on biogeographical, taxonomical and morphological studies. The results of the investigations of this thesis are organized following the three mentioned lines.

Main contributions

Biogeography

In the biogeographical context, one of our main objectives was to carry on for the first time an intensive sampling of the meiobenthos along the coast of the Iberian Peninsula in order to determine species

composition and distribution of the cyclorhagid kinorhynchs. As a result of these sampling campaigns, we could provide the first homogenized data set of all available records of cyclorhagid kinorhynchs in the Iberian Peninsula coasts gathering our results with the previous reports from the area. In total, more than half of the current cyclorhagid genera (9 out of 17) and 24 species were reported along the Iberian coasts, not observing significant differences between Atlantic and Mediterranean diversity. However, our samples were biased towards Atlantic localities and the results might not be very conclusive until more extensive sampling coverage is accomplished in Mediterranean localities. Additionally, the biological and geographical frontier between the Atlantic Ocean and the Mediterranean Sea does not seem to be coincident. Most southern "mediterranean" localities selected for our study have a big influence of Atlantic waters which enter the Mediterranean through a strong surface current. Therefore their kinorhynch fauna is more similar with the fauna described for Atlantic localities rather than for Mediterranean ones.

Kinorhynchs are part of the permanent meiofauna lacking dispersal stages and thus being assumed to have a limited dispersive potential. However, the wide distribution showed by some genera and species leads to a paradox. The mechanisms of dispersion underlying these distributional patterns are not conclusive and still remain debated. Nevertheless, some distribution patterns could be related to geological events such as the formation of the current Mediterranean Sea. This is the case of two species of *Meristoderes: M. macracanthus* from the Mediterranean Sea and *M. boylei* from the Atlantic coast of Florida. Locally, we identified potential associations between species and different kinds of benthic habitats mostly related with the type of sediment such as *Dracoderes gallaicus* with the fine mud and *Antygomonas* species with coarse sediments. Depth associations are suggestive but can be influenced by the sampling, biased towards shallow waters.

Additional explored areas yielded high kinorhynch biodiversity, being Florida coasts the most diverse with 12 cyclorhagid species belonging to 7 different genera, followed by Naples with 7 species of three genera, and Panama with 5 species of three genera. However, these data might not reflect the true diversity of the area. Although Florida and Naples are historically well sampled areas both have revealed new species demonstrating that still hold undiscovered kinorhynch diversity. This reflects the current lack of knowledge of the diversity of kinorhynchs which warns us about the still limited sampling and the need of extensive collections.

Taxonomy

As a result of the multiple samplings carried out in the Iberian Peninsula and additional areas 25 new species and one genus were discovered, 8 of these species were described whereas the remaining 17 still await formal description. The described species belong to 5 different genera, four of them containing less than 5 species, consequently the contribution to the knowledge of these genera was significant. The descriptions of the new taxa benefit from the combination of Light microscopy (LM) and Scanning electron microscopy (SEM) techniques allowing a better accuracy. Consequently, many cuticular structures not previously detected or overlooked with LM, such as the additional row of spinoscalids in the introvert of *Fissuroderes sorenseni*, the different size of the outer oral styles in several cyclorhagid genera, or the nephridial pores in *Dracoderes*, were discovered with SEM becoming relevant at taxonomic and phylogenetic levels. With the combined use of these techniques it was also possible to describe new characters, such as the different regions of the posteriormost part of each segment, in order to standardize and improve precision in future taxonomic descriptions.

Morphology

The use of confocal laser scanning microscopy (CLSM) adds a new dimension to our understanding of the organization of fundamental organ systems and their evolution in animals. The musculature and the nervous system are the only internal and segmental organ systems in kinorhynchs, therefore they seem to be important in the study of the evolution of segmentation within Ecdysozoa. Consequently, a comparative study was carried out in some cyclorhagid taxa combining cytochemical and immunocytochemical techniques in conjunction with CLSM and 3D rendering. These techniques are non-invasive and allow the examination of the specimens in whole mounts, which is an advantage to study the organs systems in detail.

Kinorhynchs are highly flexible animals able to perform multiple movements through the sediment due to the presence of a burrowing introvert and an articulated trunk divided into cuticular plates, all controlled by a complex array of muscles. *Echinoderes*, which is the most specious genus within Kinorhyncha, was selected as a model to carry on a comparative multi-species study of the myoanatomy. Results of the study showed that the musculature of different species of *Echinoderes* seems to be highly conserved in contrast with the great variability of cuticular features exhibited. Minor differences among species were only present in extremely complex regions such as the pharynx and head. The detailed description of the myoanatomy in *Echinoderes* served as a model to compare the musculature of other genera and species within the phylum. Such comparisons pointed out that all cyclorhagid kinorhynchs seem to have similar kinds and arrangements of trunk muscles. However, main and relevant differences are expected in the anteriormost trunk segments and the neck due to the different trunk closing systems. Moreover, differences also exist in the musculature associated with the midterminal spines of the posterior end, just present in some cyclorhagid genera.

Comparisons of the musculature among scalidophorans (kinorhynchs, loriciferans and priapulids) are only possible at the head and neck regions due to the obvious differences in the size and body plans of the trunk in the three groups. All three groups show well developed and combined circular and longitudinal muscles within the introvert, however their number and arrangement differ greatly in each phylum. All kinorhynchs, priapulids and loriciferans are burrowers which can explain the development of a radial symmetry in their anterior ends superimposed to the bilateral body plan that could be more an adaptation to their habitats rather than an inheritance from a common ancestor. Whether this feature is a synapomorphy of the group or just a case of functional convergence is still unresolved. More studies involving additional organs systems are necessary in order to shed light on the relationships among these groups because the musculature might be influenced by strong habitat constraints. Despite the differences in number and arrangement of muscles, the myoanatomy of kinorhynchs is comparatively more similar to loriciferans rather than to priapulids.

In order to initiate and standardize the application of immunocytochemical and CLSM techniques to the study of the nervous system in cyclorhagid kinorhynchs, we established a working protocol for labeling molecular components with standard markers such as antibodies against serotonin neurotransmitter molecules. Antibodies are relatively big molecules that cannot diffuse well through the cuticle, therefore it was necessary to make micro-incisions (trying to minimize the tissue damage) of the posterior end of the body allowing antibodies to migrate internally. Results showed high levels of anti-serotonin-like immunoreactivity in the brain and the ventral nerve cord in the three studied genera of kinorhynchs. Additional studies using antibodies against alpha tubulin helped to obtain a more complete insight of the nervous system architecture in kinorhynchs. The main differences among the nervous systems of the genera under study were related with the configuration of the neuropil, the ventral nerve cord and associated somata. Further studies including and

combining additional markers are necessary in order to detect possible patterns that give some hints of the evolution of characters among kinorhynchs.

Resumen

Generalidades

El filo Kinorhyncha está compuesto por invertebrados marinos microscópicos de vida libre, exclusivamente meiobentónicos, cuyo tamaño oscila entre 0,1 y 1 mm. Forman parte de la meiofauna, definida como el conjunto de organismos bentónicos con tamaños intermedios entre la macrofauna y microfauna, es decir, que quedan retenidos por una malla de 40-60 µm pero atraviesan una de 1 mm. Los kinorrincos se encuentran ampliamente distribuidos y se han citado desde regiones polares hasta zonas tropicales y desde la zona intermareal hasta profundidades abisales (máx. 7800 m). Habitan principalmente en los 2-3 cm superiores de sedimentos blandos, restringidos por la disponibilidad de oxígeno y de alimento. Sin embargo, pueden ocupar otros hábitats, como algas, piedras o la superficie de otros animales (bivalvos, poliquetos, briozoos, esponjas, etc.). Los kinorrincos se alimentan de detritos, algas, bacterias y diatomeas, aunque se carece de datos específicos sobre su dieta. Se han citado como fuente de alimento de moluscos bivalvos, peces de la familia Góbidos, poliquetos y gambas, y es probable que puedan aparecer en los tractos digestivos de otros animales detritívoros, aunque no existen pruebas directas o concluyentes.

El cuerpo de los kinorrincos está dividido en tres regiones: cabeza, cuello y tronco. La cabeza se compone de un introverto eversible y un cono bucal protrusible. Ambas estructuras pueden retraerse en el interior del tronco y están implicadas en la alimentación y el movimiento del animal. El cono bucal lleva cuatro anillos de apéndices cuticulares que se disponen según una simetría pentarradial. El introverto muestra numerosos apéndices cuticulares denominados escálidas, de función locomotora y sensorial, dispuestos radialmente en hasta siete anillos concéntricos. El cuello está formado por un conjunto de placas cuticularizadas denominadas plácidas que se articulan directamente con el primer segmento del tronco y funcionan como un sistema de cierre cuando la cabeza se retrae en el interior de aquél. El tronco de los kinorrincos adultos y del último estado juvenil está compuesto por 11 segmentos articulados, conectados entre sí mediante articulaciones intersegmentarias de cutícula blanda, lo que dota al cuerpo de una gran flexibilidad. Cada segmento del tronco puede tener bien forma anular o estar subdividido longitudinalmente en hasta cuatro placas: dorsal (tergal) y ventrales (esternales), articuladas entre sí. El tronco presenta gran diversidad de estructuras cuticulares distribuidas segmentariamente, como espinas, salientes espinosos, túbulos, sedas, fosetas sensoriales, salidas glandulares y pelos cuticulares. Todas estas estructuras muestran una gran diversidad morfológica entre especies y géneros, por lo que constituyen importantes caracteres diagnósticos. De hecho, el tipo y disposición de estas especializaciones cuticulares es generalmente exclusivo en el nivel especie. La segmentación de los kinorrincos es evidente en el exterior del cuerpo; sin embargo, no todos los sistemas orgánicos internos reflejan el mismo patrón anatómico. Los músculos, los nervios y las glándulas muestran un cierto patrón segmentario, que no siguen los órganos digestivo, reproductor y excretor. No existen sistemas respiratorio ni circulatorio.

Filogenéticamente los Kinorrincos se sitúan dentro de los Ecdisozoos, unos de los superclados de Protóstomos que incluye a artrópodos, tardígrados, onicóforos, nematodos, nematomorfos, loricíferos y priapúlidos. Dentro de los Ecdisozoos, los kinorrincos están emparentados con nematomorfos, nematodos,

priapúlidos y loricíferos en el clado Cicloneuralios, todos ellos relacionados por la posesión de un cerebro anular alrededor de la faringe. Dentro de los cicloneuralios, los morfólogos también han agrupado a kinorrincos, priapúlidos y loricíferos bajo la denominación Escalidóforos por la posesión de un introverto con escálidas y la presencia de dos anillos de músculos retractores en el introverto. La mayoría de los estudios filogenéticos sitúan a los kinorrincos como grupo hermano de los priapúlidos, ambos situados en un posición basal dentro de los Ecdisozoos; mientras que la posición de los loricíferos permanece menos clara. La filogenia interna de los kinorrincos, está todavía por resolver, aunque en algunos estudios recientes ya se han comenzado a incluir datos moleculares.

Hasta la fecha el filo Kinorrincos comprende 210 especies (basadas en descripciones de animales adultos) agrupadas en 23 géneros. Durante los últimos 10 años el número de especies y géneros descritos se ha incrementado considerablemente, lo que significa que gran parte de la biodiversidad del filo está aún por descubrir. Esta Tesis se centra en el estudio de uno de los dos órdenes actuales dentro del filo, los Ciclorrágidos. Se trata del orden más diverso y más ampliamente distribuido, con 17 géneros y hasta 130 especies. Los kinorrincos ciclorrágidos se caracterizan por tener un cuello compuesto por 16 plácidas, el primer segmento del tronco en forma de anillo y de sección ovalada, y por la presencia de espinas cuticulares a lo largo del tronco.

Los estudios en el filo Kinorhyncha: estado actual

En la actualidad, la comunidad internacional de investigadores dedicados al estudio de los kinorrincos no supera las 10 personas, siendo la mayoría discípulos o colaboradores de Robert P. Higgins, uno de los científicos más destacados y con mayores aportaciones al filo. A lo largo de los años otros muchos científicos no especialistas también han realizado contribuciones al conocimiento del filo de manera esporádica, la mayoría de las veces en colaboración con los primeros.

Actualmente, las investigaciones en el filo Kinorhyncha están focalizados en el estudio de áreas inexploradas para describir nuevos taxones, así como en el estudio de la morfología a través de técnicas modernas como la microscopía confocal láser. A lo largo del tiempo, y con el perfeccionamiento de técnicas de microscopía, los caracteres taxonómicos significativos y la terminología aplicada a los kinorrincos ciclorrágidos se han ido modificando con la inclusión de nuevos términos que han permitido descripciones más precisas y específicas.

Por otro lado, nuevos estudios filogenéticos comienzan a incorporar datos moleculares, aunque todavía necesitan ser mejorados para conseguir más resolución. Desafortunadamente, estudios embriológicos y de desarrollo no han podido ser llevados a cabo con éxito en el filo debido a las dificultades presentadas para poder cultivar y mantener a los animales. Desde los estudios iniciados por Remane, que describió el desarrollo directo sin fases larvarias dispersivas, seguidos por Nyholm y Kozloff que consiguieron estudiar el ciclo vital completo y observar el desarrollo postembrionario, no se han obtenido nuevos datos.

En la actualidad la información existente sobre la distribución geográfica de lo kinorrincos ciclorrágidos es extremadamente fragmentada, siendo un mero reflejo de las actividades de los pocos expertos que han estado estudiando el filo a lo largo del tiempo. La Península Ibérica fue un área casi inexplorada en este sentido hasta finales de la década de 1990, cuando nuestro grupo de investigación inició una serie de proyectos pioneros para el estudio de los kinorrincos de las costas españolas, financiados por el Plan Nacional de Investigación y Desarrollo. Esta tesis doctoral se gestó en el marco de éstas investigaciones incluyendo además estudios realizados en otras áreas.

Objetivo principal

En el contexto previo, queda claro que los estudios sobre los kinorrincos ciclorrágidos están aún en etapas iniciales. Existe una gran carencia de conocimientos en muchas áreas de estudio como la taxonomía, la biogeografía, la ecología o la morfología mientras que otras están solamente comenzando a ser exploradas, como la filogenia molecular o la biología del desarrollo. De acuerdo con esta situación, el objetivo general de esta Tesis es contribuir a la integración de tres puntos de vista diferentes (biogeográfico, taxonómico y morfológico) para incrementar los conocimientos actuales sobre los kinorrincos ciclorrágidos con una perspectiva amplia. Los resultados derivados de las investigaciones realizadas durante el desarrollo de esta tesis se agrupan en base a las tres líneas mencionadas anteriormente.

Aportaciones principales

Biogeografía

En el contexto biogeográfico, nuestro principal objetivo fue desarrollar por primera vez una campaña intensiva de muestreos meiobentónicos a lo largo de las costas españolas para determinar la composición de la fauna de kinorrincos ciclorrágidos y su distribución. Como resultado de estas campañas se ha obtenido el primer conjunto de datos que abarca todos los registros obtenidos hasta la fecha en la Península Ibérica. En total se han registrado más de la mitad de los géneros actuales de ciclorrágidos (9 de 17), con 24 especies, sin que se hayan observado diferencias significativas entre la diversidad de las costas atlánticas y las mediterráneas. Sin embargo, nuestros muestreos están sesgados, siendo más intensos en las localidades del Atlántico, por lo que los resultados totales pueden no ser totalmente concluyentes hasta que se lleven a cabo con el mismo esfuerzo de muestreo en aguas mediterráneas. Además, las fronteras geográfica y biológica entre el Atlántico y el Mediterráneo no parecen ser coincidentes. La mayoría de las localidades estudiadas situadas en el Mediterráneo Sur tienen una gran influencia de aguas atlánticas que penetran en el Mediterráneo a través de fuertes corrientes superficiales, lo que determina una fauna de kinorrincos más similar a las especies de aguas atlánticas que a las mediterráneas.

Los kinorrincos son parte de la meiofauna permanente careciendo de estados dispersivos, por lo que se les atribuye un potencial de dispersión muy limitado. Sin embargo y paradójicamente, algunos géneros y especies muestran distribuciones geográficas muy amplias. Los mecanismos de dispersión que subyacen a estos patrones de distribución son aún desconocidos y objeto de debate. No obstante, alguno de estos patrones puede estar relacionado con procesos geológicos, como la formación del actual Mar Mediterráneo. Es el caso de dos especies del género *Meristoderes*: *M. macracanthus* del Mediterráneo y *M. boylei* de la costa atlántica de Florida. A escala local, hemos identificado asociaciones potenciales entre las especies y los distintos hábitats bentónicos, principalmente relacionadas con el tipo de sedimento, como ocurre en *Dracoderes gallaicus* con el fango fino y varias especies de *Antygomonas* con los sedimentos gruesos. Las preferencias por distintas profundidades son en principio interesantes, aunque los datos aún adolecen de muestreos insuficientes, restringidos a aguas poco profundas.

Otras zonas del mundo muestreadas produjeron también una elevada biodiversidad de kinorrincos. La costa de la península de Florida resultó ser la más diversa, con 12 especies de ciclorrágidos pertenecientes a 7 géneros diferentes, seguida por la bahía de Nápoles con 7 especies de tres géneros, y Panamá, con 5 especies de tres géneros. Sin embargo, estos datos probablemente no reflejen la diversidad total de estas zonas. Aunque Florida y Nápoles son áreas históricamente bien muestreadas, en ambos casos han aparecido

nuevas especies lo que demuestra que aún albergan diversidad oculta. Esto pone de manifiesto las lagunas en el conocimiento de la diversidad del filo y la necesidad de muestreos sistemáticos.

Taxonomía

Como resultado de los múltiples muestreos llevados a cabo en la Península Ibérica y en las áreas adicionales se ha descubierto un nuevo género y 25 nuevas especies, de las que se han descrito 8, mientras que las 17 restantes esperan una descripción formal. Las especies descritas pertenecen a cinco géneros distintos, cuatro de los cuales comprenden menos de cinco especies cada uno, por lo que la contribución al conocimiento de dichos géneros puede calificarse de significativa. Las descripciones de los nuevos taxones se han efectuado combinando técnicas de microscopía óptica (LM) y electrónica de barrido (SEM), lo que permite una mayor precisión. En consecuencia, muchas estructuras cuticulares desconocidas o desapercibidas con LM quedaron de manifiesto con SEM y resultaron de relevancia taxonómica y filogenética. Es el caso del círculo adicional de espinoscálidas en el introverto de *Fissuroderes sorenseni*, el diferente tamaño de los estiletes orales en distintos géneros de ciclorrágidos, o los conspicuos poros nefridiales de *Dracoderes*. Con el uso combinado de estas técnicas de microscopía también ha sido posible la descripción de nuevos rasgos, como la caracterización del borde posterior de cada segmento y su respectiva región articular, que permiten estandarizar y dotar de mayor precisión futuras descripciones taxonómicas.

Morfología

La utilización de la microscopía láser confocal (CLSM) añade una nueva dimensión al conocimiento de la organización de los sistemas orgánicos y su evolución en los animales. La musculatura y el sistema nervioso son los únicos sistemas orgánicos internos con organización segmentaria en los kinorrincos, por lo que pueden resultar de gran interés para el estudio de la evolución de la segmentación en los Ecdisozoos. En consecuencia, se ha llevado a cabo un estudio comparado en varios taxones de ciclorrágidos mediante el uso combinado de técnicas citoquímicas e inmunocitoquímicas junto con CLSM y reconstrucciones tridimensionales computarizadas. Estas técnicas no son invasivas y permiten examinar los ejemplares *in toto*, lo que representa una gran ventaja para el estudio detallado de los sistemas orgánicos.

Los kinorrincos son animales dotados de gran flexibilidad, capaces de realizar múltiples desplazamientos a través del sedimento gracias a los movimientos del introverto y a su tronco articulado dividido en múltiples placas, todo ello controlado y manejado por un complejo conjunto de músculos. El género *Echinoderes*, que contiene el mayor número de especies dentro del filo, se seleccionó como modelo para llevar a cabo un estudio comparado multiespecífico de la anatomía muscular. Los resultados de dicho estudio han mostrado que la musculatura de las diferentes especies de *Echinoderes* es muy homogénea, lo que contrasta con la enorme variabilidad de rasgos cuticulares entre especies. Solamente aparecieron pequeñas diferencias interespecíficas en regiones extremadamente complejas, como la faringe y la cabeza. La descripción detallada de la mioanatomía en *Echinoderes* se ha utilizado como modelo para comparar la musculatura de otros géneros y especies del filo. Estas comparaciones han demostrado que todos los ciclorrágidos parecen poseer tipos y disposiciones similares de músculos en el tronco. Sin embargo, se esperan grandes y significativas diferencias en los primeros segmentos y en el cuello, asociados con los distintos sistemas de cierre que presentan diversos taxones. Además también existen diferencias en la musculatura asociada a las espinas medioterminales del extremo posterior del cuerpo, que solo existen en determinados géneros de ciclorrágidos.

El estudio comparado de la musculatura en los Escalidóforos (Kinorrincos, Loricíferos y Priapúlidos) solo

es posible en las regiones corporales de la cabeza y el cuello. Los tres grupos presentan combinaciones de músculos circulares y longitudinales bien desarrollados en el introverto, aunque existen grandes diferencias en su número y disposición entre los tres filos. Tanto los kinorrincos como los loricíferos y los priapúlidos son animales excavadores, lo que puede explicar el desarrollo de una organización radialmente simétrica en sus extremos anteriores superpuesta al plan corporal bilateral. De ser así, la presencia de un introverto radial sería más una adaptación al hábitat que una herencia de un antepasado común. Por ello, la consideración de este rasgo como una sinapomorfía del grupo o como un caso de convergencia funcional es todavía objeto de debate. Se hace necesario realizar nuevas investigaciones en otros sistemas orgánicos para arrojar luz sobre las relaciones entre estos grupos, dado que la musculatura puede verse influida por fuertes condicionantes ambientales. A pesar de las diferencias en el número y disposición de los músculos, la anatomía muscular de los kinorrincos es comparativamente más próxima a la de los loricíferos que a la de los priapúlidos.

Con el objeto de iniciar y estandarizar la aplicación de las técnicas inmunocitoquímicas y de CLSM al estudio del sistema nervioso de los kinorrincos ciclorrágidos, hemos establecido un protocolo de trabajo para diferenciar específicamente componentes moleculares con marcadores estandarizados, como los anticuerpos antiserotonina. Los anticuerpos son moléculas relativamente grandes que no difunden bien a través de la cutícula, lo que hace necesaria la realización de microincisiones (tratando de minimizar el daño tisular) en el extremo posterior del cuerpo para permitir su migración interna. Los resultados muestran altos niveles de inmunorreactividad antiserotonina en el cerebro y en el cordón nervioso ventral en los tres géneros de kinorrincos estudiados. Mediante estudios adicionales con anticuerpos antitubulina alfa se ha obtenido una apreciación más completa de la arquitectura nerviosa de los kinorrincos. Las principales diferencias en los sistemas nerviosos de los géneros estudiados se refieren a la configuración del neuropilo, el cordón nervioso ventral y los cuerpos celulares asociados. Las investigaciones realizadas son preliminares y necesitan ser completadas, por lo que son necesarias nuevas investigaciones con diferentes marcadores y combinaciones de estos para detectar posibles patrones que puedan contribuir a la comprensión de la evolución de determinados rasgos dentro del filo.

List of included publications

The core of this Doctoral Thesis is composed of seven publications included in the Results section, each one defining one Chapter, and two Appendixes to Chapters I and VII with complementary unpublished material.

- **Chapter I**

  Sánchez N, HERRANZ M, Benito J, Pardos F (2012). The phylum Kinorhyncha in Spain: new data from the first intensive sampling campaigns. *Zootaxa* 3402: 24–44.

  Appendix I

  Additional data from collections after 2011

- **Chapter II**

  HERRANZ M, Thormar J, Benito J, Sánchez N, Pardos F (2012). *Meristoderes* gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae). *Zoologischer Anzeiger* 251, 161–179.

- **Chapter III**

  Sørensen MV, HERRANZ M, Rho HS, Min WG, Yamasaki H, Sánchez N, Pardos F (2012). On the genus *Dracoderes* Higgins & Shirayama, 1990 (Kinorhyncha: Cyclorhagida) with a redescription of its type species, *D. abei*, and a description of a new species from Spain. *Marine Biology Research* 8: 210–232.

- **Chapter IV**

  HERRANZ M, Sánchez N, Pardos F, Higgins RP (2014). New Kinorhyncha from Florida coastal waters. *Helgoland Marine Research* 68: 59-87.

- **Chapter V**

  HERRANZ M, Pardos F (2013). *Fissuroderes sorenseni* sp. nov. and *Meristoderes boylei* sp. nov.: First Atlantic recording of two rare kinorhynch genera, with new identification keys. *Zoologischer Anzeiger* 253: 93–111.

- **Chapter VI**

  HERRANZ M, Pardos F, Boyle MJ (2013). Comparative morphology of serotonergic-like immunoreactive elements in the central nervous system of kinorhynchs (Kinorhyncha, Cyclorhagida). *Journal of Morphology,* 274: 258–274.

- **Chapter VI**

  HERRANZ M, Boyle MJ, Pardos F, Neves RC (submitted). Comparative myoanatomy of *Echinoderes* (Kinorhyncha): A comprehensive investigation by CLSM and 3D reconstruction. *Frontiers in Zoology*.

  Appendix II

  New data on the neuroanatomy of cyclorhagid kinorhynchs through immunocytochemistry and CLSM

Arrangement of Figures

Figures are arranged and numbered by section (1 for Introduction, 2 for Material and Methods, and so on). Appendix figures are preceded by A.I. (Appendix I) or A.II. (Appendix II). Figures inside the publications (Chapters I to VII) are independent from this system and follow the arrangement and style of their respective journals.

Cover image: depth code image of the head of *Tubulideres seminoli,* apical view

Inner cover images:

Biogeography, maximum projections of the autofluorescent cuticle of different species of cyclorhagid kinorhynchs.

Taxonomy, maximum projection of the autofluorescent cuticle of *Echinoderes horni*, lateral view.

Morphology, maximum projection of the autofluorescent cuticle and the stained musculature of *Echinoderes horni*, lateral view.

How inappropriate to call this planet Earth when it is quite clearly Ocean

Arthur C. Clarke

1 Introduction

1.1. What is a kinorhynch?

Who has never taken a handful of sand on the beach? At first glance, one might view it as an infertile and depauperate habitat. Such a view is undoubtedly biased by the scale at which humans operate. Imagine a whole world in a grain of sand inhabited by tiny representatives of most of Earth's major animal groups (23 out of the 35 extant animal phyla) where the largest specimen is smaller than the period at the end of this phrase. This group of animals is called meiofauna, a word that refers to their small size which is intermediate between macro and microfauna (Fig. 1.1). Despite its diversity and wide distribution in the marine and freshwater environment, the meiofauna is easily overlooked and therefore poorly appreciated. However, these minute organisms are far more important ecologically than is generally known and it is becoming clearer and clearer that we cannot intend to understand animal evolution without paying attention to them (Garey and Schmidt-Rhaesa 1998).

One of these minor and bizarre understudied groups of the meiofauna are kinorhynchs, common named mud dragons and subject of the present thesis. Kinorhyncha is defined as a phylum of microscopic free-living marine invertebrates exclusively meiobenthic ranging in size from 0.1 to 1mm (Higgins and Thiel 1988; Kristensen and Higgins 1991). The scientific name Kinorhyncha is derived from the Greek *kīn(ē)- (κινέω)* meaning "to move" + *rhynkho (ῥύγχος)* meaning "snout" (animals with a motile snout) making reference to the characteristic in-and-out movement of the retractable head performed by live specimens (Reinhard 1885, Giere 2009).

The first kinorhynch was observed in 1841 by the French scientist Felix Dujardin who called it "L'Echinodère" and classified it as something intermediate between worms and crustaceans (Giere 2009). An alternative name "Kinorhyncha (Echinoderes)" was posteriorly proposed by Reinhard in 1885. However, Zelinka in his monograph (1928) did not use it and referred to the Kinorhyncha as "Echinodera", latinized from Dujardin's original name which derives from the Greek *ekhīno- (ἐχῖνος)* meaning "hedgehog" and *deres (δεῖρος)* meaning "neck". These terms refer to the spiny scalids misinterpreted as part of the neck by Dujardin in 1851 (Neuhaus 2013). Nowadays, the scientific name for the group is Kinorhyncha, as suggested by Reinhard, whereas *Echinoderes* represents the first genus described within the phylum (Fig. 1.2).

Fig. 1.1. Representation of the marine meiofauna from Giere (2009). Kinorhyncha represented down in the left.

Fig. 1.2. LM micrograph of *Echinoderes spinifurca*, introvert and mouth cone extended, lateral view, anterior is to the left. Note the presence of a red eyespot in the first row of spinoscalids of the introvert and the orange color of the glands along the trunk and posteriormost segments.

1.2. Ecological aspects

Kinorhynchs are purely marine and widely distributed members of the meiofauna, this latter considered as the group of benthic animals that get retained in a mesh of 60-40 µm but pass through a 1mm mesh (Higgins and Thiel 1988; Giere 2009). They have been reported from polar to tropical regions and from the intertidal to abyssal depths (max. 7800 m Danovaro et al. 2002). Kinorhynchs are mostly found in the upper 2-3 cm centimeters of the muddy sediment, their presence and abundance are influenced by the oxygen availability and food supply (Kristensen and Higgins 1991). Although they normally occupy the upper layer of fine sediments they have also been found interstitially reaching 40-70 cm depth in coarse sand beaches where the water of the interstices is well oxygenated (Kristensen and Higgins 1991; Sørensen 2008a). Despite the sediment is the preferred habitat for most kinorhynchs, they have been reported occupying other habitats such as algae, stones, or the surface of different metazoans (bivalves, polychaetes, bryozoans, sponges, etc.) but always associated with the small fraction of sediment somehow deposited on these different surfaces (Higgins 1977, 1978, 1986; Higgins and Thiel 1988; Kristensen and Higgins 1991; Sørensen and Pardos 2008).

Kinorhynch abundance is greatly variable ranging from a few up to 50 specimens per cm^2. However, some studies in Polar Regions yielded even higher abundances reaching more than 250 specimens per cm^2 and hence representing 5-6% of the meiobenthic fauna (Higgins and Thiel 1988; Neuhaus and Higgins 2002).

Although kinorhynchs are strictly marine organisms some species are able to tolerate a wide range of salinities and temperature for instance, *Echinoderes levanderi* Karling, 1954 can tolerate salinities down to 6-7 ‰ in the Baltic Sea (Karling 1954) whereas *Echinoderes coulli* Higgins, 1977 can survive a range from 12 to 42 ‰ in an intertidal flat in North Carolina (Horn 1978). High temperatures could be a limiting factor for the presence of kinorhynchs being 33°C the maximum registered for species of *Pycnophyes* and *Echinoderes* in a lagoon in India (Rao and Satapathy 1996). By contrast low temperatures do not seem to be a problem, the lowest documented temperature was −1.85° C at 385 m depth for *Campyloderes vanhoeffeni* Zelinka, 1913 in the Antartic (Zelinka, 1913).

Kinorhynchs seem to graze on detritus, algae, bacteria and diatoms but specific data about their feeding is still scarce. Zelinka (1928) reported that kinorhynchs from deep waters might ingest sediment with detritus, that contain high amounts of bacteria (Neuhaus 1991, Neuhaus 2013 and references herein), whereas kinorhynchs from the photic zone might consume considerable amounts of algae and diatoms (Fig. 1.3). Several authors described diatoms as part of the gut content of different kinorhynchs (Kozloff 1972; Higgins 1990; Neuhaus 1993), whether this diatoms were actively or erroneusly ingested is still uncertain. Moreover, Brown (1985) speculated that kinorhynchs might excret mucus in order to provide a substrate to culture bacteria, diatoms or other ectocommensals. Different feeding mecanisms have been hypothesized for the cyclorhagid genus *Echinoderes* that can selectively suck algae protruding the pharynx or use the introvert and terminal spines to trap them (Adrianov and Malakhov 1994). Furthermore, the presence of bacteria associated with the gut within cells or vesicles was speculated to be endosymbiotic, suggesting an addittonal source of nutrients (Brown 1985).

Kinorhynchs have been reported as food source of bivalve molluscs, fish of the family gobiidae polychaetes and shrimps (Martorelli and Higgins 2004; Neuhaus 2013 and references herein). Three new kinorhynch species of three different genera were described from the stomach of the Argentine red shrimp (Martorelli and Higgins 2004). Indeed, it is most likely that they can also appear in the digestive tracts of other deposit-feeding animals, but no direct evidence has yet been reported.

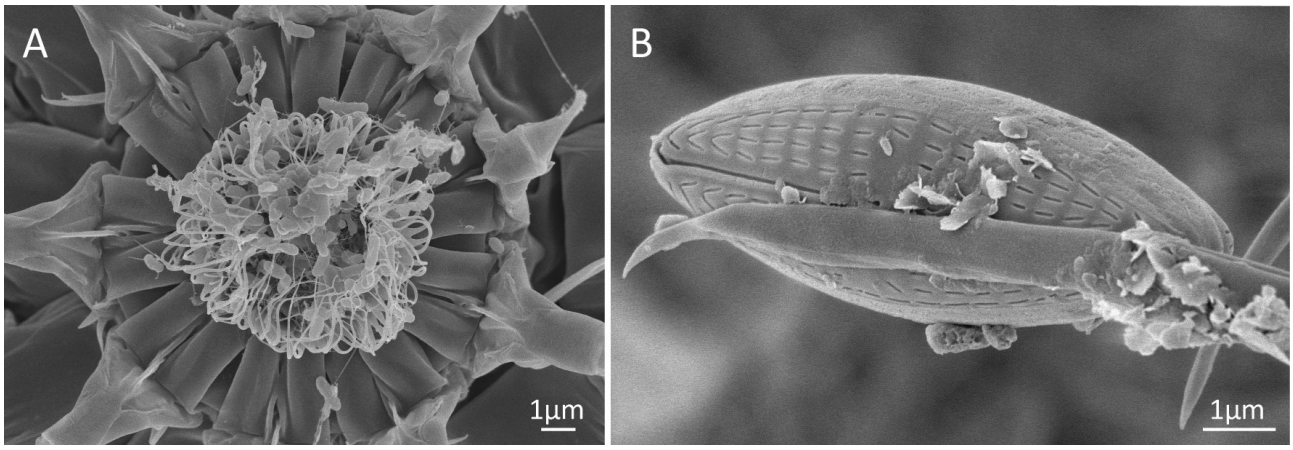


Fig. 1.3. SEM micrographs of putative kinorhynch food. (A) Detail of the mouth cone of *Echinoderes spinifurca* ingesting bacteria. (B) Diatom stuck to one introvert spinoscalid in *Antygomonas paulae.*

1.3. Anatomy

External anatomy

The body of kinorhynchs is divided into three regions: head, neck and a trunk composed of 11 articulated segments (Fig. 1.4. A-B). Traditionally the head and neck were considered “segments” (head=segment 1, neck=segment 2, first trunk segment=segment 3) raising the total number to 13. This terminology was established by Zelinka (1928) based on the consideration of kinorhynchs being strictly segmented animals which was used afterwards in all studies until Neuhaus and Higgins (2002). The latter study proposed a new segment numbering from 1 to 11 excluding the head and neck based on the absence of segmental internal and external characters (Neuhaus and Higgins 2002). This numbering was assumed and used in all modern studies where the first trunk segment is segment 1.

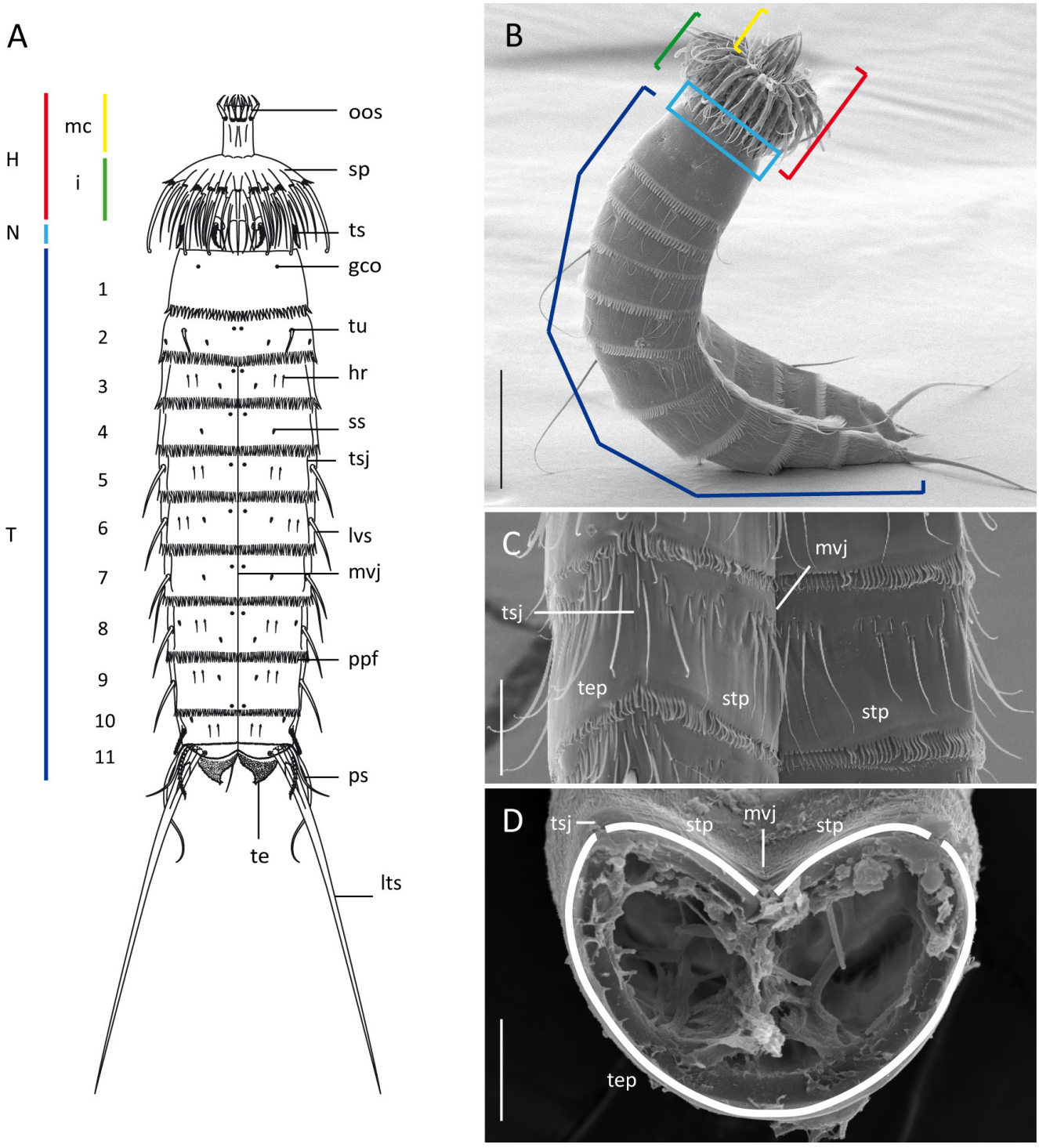


Fig. 1.4. General kinorhynch morphology. (A) Schematic of a standard cyclorhagid male, the most relevant morphologic characters are labelled, ventral view, anterior is up. (B) SEM micrograph of a cyclorhagid kinorhynch showing the main parts of the body, lateral view, anterior is up. (C) SEM micrograph of the ventral side of trunk segment 7 in *Meristoderes*. (D) SEM micrograph of a cross section of the trunk in *Echinoderes* showing the division of the segment in two sternal (ventral) and one tergal (dorsal) plate, ventral is up. Numbers refer to segments. Abbreviations: gco, glandular cell outlet; h, head; hr, cuticular hair; i, introvert; lts, lateral terminal spine; lvs, lateroventral spine; mc, mouth cone; mvj, midventral joint; n, neck; oos, outer oral style; ppf, primary pectinate fringe; ps, penile spine; ss, sensory spot; sp, spinoscalid; stp, sternal plate; t, trunk; te, tergal extension; tep, tergal plate; ts, trichoscalid; tsj, tergosternal joint; tu, tubule. Scale bars B: 50 µm, C-D: 10 µm.

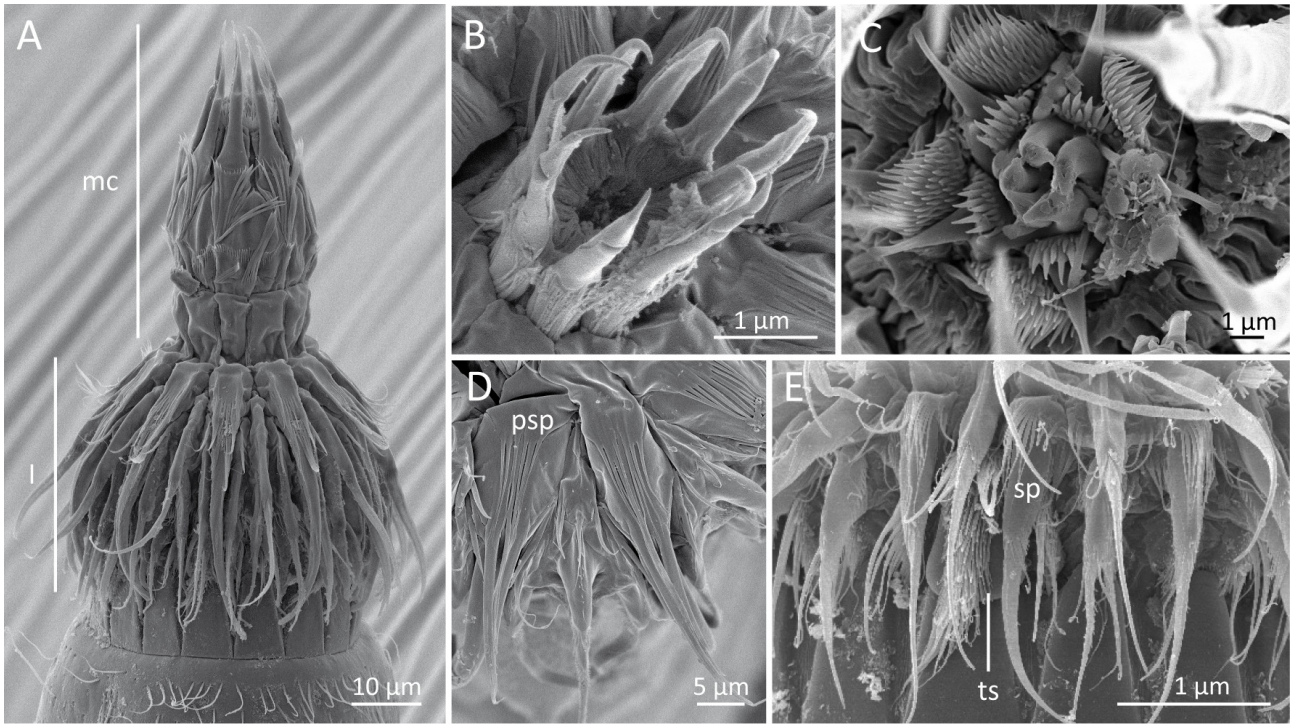


Fig. 1.5. Kinorhynch head, SEM micrographs. (A) Head showing the mouth cone and the introvert in *Echinoderes*. (B) Detail of the outer oral styles of the mouth cone in *Tubulideres*. (C) Detail of the inner oral styles of the mouth cone in *Antygomonas*. (D) Detail of the primary spinoscalids of the introvert in *Tubulideres*. (E) Detail of the spinoscalids and trichoscalids of the introvert in *Echinoderes*. Abbreviations: i, introvert; mc, mouth cone; sp, spinoscalids; psp, primary spinoscalids; ts, trichoscalids.

Sometimes in the literature the term “zonite” has been utilized instead of “segment”; this was suggested by Zelinka (1928) to somehow differentiate the segmentation of kinorhynchs in comparison with that in arthropods and annelids. However the term “zonite” has scarcely been used in the literature (e.g., Higgins 1960; Nebelsick 1990; Bauer-Nebelsick 1995, 1996) being the term segment the most extended and currently adopted by most authors (Neuhaus 2013). This does not imply an assumed homology among the segments of kinorhynchs, arthropods and annelids (Neuhaus 2013).

Head

The head of a kinorhynch is composed of an eversible introvert and a protrusible terminal mouth cone (Fig. 1.5. A). Both structures can be retracted into the trunk and are involved in the feeding and motion of the animal.

The mouth cone carries four rings of cuticular appendages usually arranged following a pentaradial symmetry. The most prominent of these appendages are the outer oral styles, typically nine, which define ring 00 (Fig. 1.5. B). The structure and size of these oral styles vary among species and genus and is often used as a taxonomic character. Inside the mouth cone there are three further rings of inner oral styles (-01, -02, -03) that are only visible under forced unnatural protrusion, e.g. during fixation or due to freshwater shocking (Brown 1989; Nebelsick 1993; Neuhaus 1994; Sørensen and Pardos 2008) (Fig. 1.5. C).

The introvert bears several cuticular sensory and locomotory appendages, named scalids, arranged radially and staggered in up to seven concentric rings (Neuhaus and Higgins 2002; Sørensen and Pardos 2008). Depending on the species and genus each ring possesses a variable number of scalids that normally do not

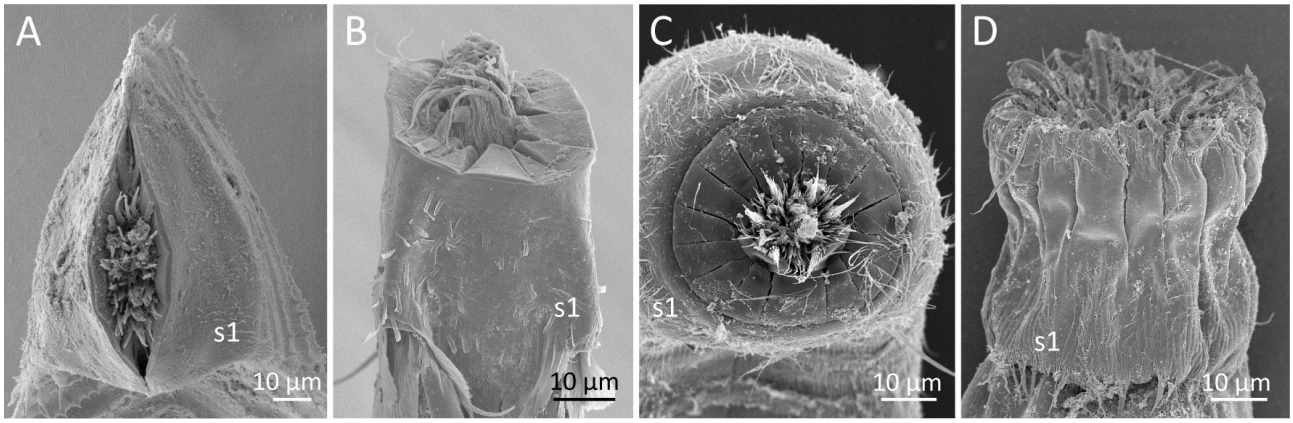


Fig. 1.6. Neck and first trunk segment in different kinorhynch genera, SEM micrographs. (A) *Semnoderes armiger* shows a bilateral closing system involving segment one (clam-shell apparatus). (B) *Antygomonas paulae* has an intermediate closing system between the bilateral and the radial. (C) *Echinoderes dujardinii* shows a radial closing system. (D) *Zelinkaderes floridensis* shows a radial closing system with the absence of articulation between the placids and the first trunk segment. Abbreviations: s1, segment one.

exceed 20. There are three types of scalids in the introvert: primary spinoscalids, spinoscalids and trichoscalids (Neuhaus 2013). The primary spinoscalids occupy the first ring of the introvert (01) and are generally elongated consisting of a wide basal and a long distal part ending in a blunt tip (Fig. 1.5. D). The spinoscalids in the following 5-6 rings (02-07) have a similar appearance being shorter and slender than the primary spinoscalids with pointed tips and usually decreasing in length posteriorly (Fig. 1.5. E). The trichoscalids are hairy and short scalids; they occupy the posterior part of the introvert usually arising from special cuticular plates that are associated to the neck region called trichoscalid plates (Fig. 1.5. F). As a consequence, trichoscalids do not follow the radially symmetrical patterns of remaining scalids and hence could be considered as part of the neck (Sørensen and Pardos 2008).

Neck

The neck is composed of a set of small and cuticularized plates called placids that generally articulate with the first trunk segment acting as a closing apparatus when the head is retracted into the trunk. The appearance and number of placids can differ among genera although there are usually 14-16 in the order Cyclorhagida and 6-8 in Homalorhagida (Sørensen and Pardos 2008). Some cyclorhagid genera such as *Semnoderes, Antygomonas* or *Zelinkaderes* possess modified first trunk segments which are also part of the closing apparatus of the animal, a feature that accounts for traditional taxonomic groupings at the order rank (conchorhagids and cryptorhagids) scarcely used today but awaiting taxonomical evaluation (Fig. 1.6. A-D).

Trunk

The trunk in adult kinorhynchs and juveniles of the last stage is composed of 11 segments (Fig. 1.4). The segments connect and articulate each other through a soft cuticular intersegmentary joint (Gª Ordóñez et al. 2000) allowing the animal to be highly flexible.

Each trunk segment can be either ring-like or subdivided longitudinally in up to four dorsal (tergal) and ventral (sternal) plates that articulate with each other. The most common organization of cuticular plates in a segment is having a single tergal (dorsal) plate and two sternal (ventral) plates articulating in lateral (tergosternal junctions) and midventral position (midsternal junction) (Fig. 1.4. C-D). The presence, absence, number and arrangement of the cuticular plates, mostly in the first and second trunk segments, play a very important taxonomic role and are crucial for the discrimination of different families and genera within Kino-

rhyncha (Sørensen and Pardos 2008).

The cuticle of every trunk segment is anteriorly thickened forming an internal structure called pachycyclus where the segmental muscles attach; the posterior end of each segment overlaps the following segment and sometimes extends in a comb-like shape called pectinate fringe. The pectinate fringe pattern varies among species and is used as a taxonomic character, however other cuticular structures are more important for taxonomic purposes.

The trunk is equipped with many different cuticular structures distributed segmentally such as spines, spinose processes, tubules, setae, sensory spots, glandular cell outlets, and cuticular hairs (Fig. 1.4). All these structures show an extraordinary morphological diversity among species and genera being very important as diagnostic characters. Indeed, the type and arrangement of these cuticular specializations is normally unique at species level.

- The spines consist of a proximal articulation and a distal end piece that can be rigid and pointy (acicular spine) or soft, flattened basally and pointy distally (cuspidate spine). Special mention deserve the lateral terminal spines, located lateroventrally on the last segment of the body in most genera. They can be very long, even reaching or exceeding the trunk length (Fig. 1.4. A).

- The spinose processes are cuticular projections without a proximal articulation.

- The setae are short and thin tubular structures with a terminal pore and blunt tip (Neuhaus 2013) mainly present in homalorhagids with an important taxonomic value at species level.

- The tubules (sometimes named adhesive tubules in traditional literature), are short and flexible tubes with a distal opening at the tip. Sometimes they show a wide base and a narrow distal part flanked by two cuticular flaps (Gª Ordóñez et al. 2000) (Fig. 1.4. A).

- The sensory spots are present in almost every segment; they are small round to oval cuticular areas, slightly depressed and normally showing one or two pores and a cilium protruding from one of the pores, all surrounded by short papillae (Nebelsick 1992a) (Fig. 1.4. A). Sometimes these structures are associated with glands (Brown 1985; Kristensen and Higgins 1991).

- The epidermal glandular cell outlets can be cuticular clusters of small perforations of different sizes or large gland openings. The glandular cells secrete a mucous-like substance that has been associated with the locomotion or the reproduction (Sørensen and Pardos 2008) (Fig. 1.4. A).

- Cuticular hairs are small and usually filiform cuticular structures that can emerge directly or through perforations of the cuticle. They can cover extensive areas of the body surface forming distinctive patterns sometimes species-specific (Fig. 1.4. A, C).

Internal anatomy

Segmentation in kinorhynchs is evident externally, however internally not all the organ systems mirror the same pattern. Muscles, nerves and glands show an apparent segmental arrangement whereas gut, reproductive and excretory organs are non-segmental (Zelinka 1928; Kristensen and Hay-Schmidt 1989; Kristensen and Higgins 1991; Gª Ordóñez et al. 2000; Neuhaus and Higgins 2002). Moreover, respiratory and circulatory systems are absent (Kristensen and Higgins 1991; Neuhaus and Higgins 2002).

The cuticle, secreted by a thin monolayered and non-ciliary epidermis, is thick, sclerotized and periodically molted to permit growth. It consists of three layers: a thin outer epicuticle, a middle intracuticle and a

fibrilar procuticle (Kristensen and Higgins 1991; Neuhaus 1994; Gª Ordóñez et al. 2000; Neuhaus and Higgins 2002; Neuhaus 2013). The chitinous three layered cuticle surrounds the entire animal including the foregut, hindgut, neprhidiopores and tubes of the sensory areas (Neuhaus 1994). The subjacent epidermis contains segmental mucous glands which open through pores in the cuticle and secrete a mucous layer over the cuticle (Brown 1985; Kristensen and Higgins 1991; Gª Ordóñez et al. 2000). This has been interpreted as a protection against the abrasion produced by the friction during the movement through the sediment (Kristensen and Higgins 1991).

The body musculature of kinorhynchs is located beneath the epidermal basal lamina and is composed of several sets of longitudinal, diagonal and dorsoventral muscles (Zelinka 1928; Kristensen and Higgins 1991) most of them following a segmental organization. Muscle fibers always attach to the cuticle through an intermediate epidermal cell (Kristensen and Higgins 1991). Circular muscles are absent in the trunk but present in the head and neck regions. Except for the muscles associated with the midgut, all the musculature is distinctly cross-striated as in arthropods (Müller and Schmidt-Rhaesa 2003; Rothe and Schmidt-Rhaesa 2004; Schmidt-Rhaesa and Rothe 2006).

The central nervous system consists of a circumenteric brain and several longitudinal nerve cords in the trunk that are connected by two commissures per segment (Zelinka 1928; Kristensen and Higgins 1991; Nebelsick 1993; Neuhaus and Higgins 2002). The brain is divided transversely into three regions consisting of anterior and posterior neuronal somata separated by a central neuropil (Kristensen and Higgins 1991; Nebelsick 1993; Neuhaus 1994; Neuhaus and Higgins 2002). This ring-like brain is shared with other cycloneuralians. The ventral nerve cord is double and extends posteriorly from the brain bearing a pair of ganglia per segment connected by commissures (Kristensen and Higgins 1991; Neuhaus and Higgins 2002; Neuhaus 2013).

The sense organs include several cuticular structures such as, scalids, mouth cone styles, spines, glands and sensory spots that seem to be associated with monociliary sensory cells (Brown 1989; Kristensen and Higgins 1991; Nebelsick 1993). Anterior, red pigmented eyes with an unusual structure are sometimes found at the level of the first ring of spinoscalids. They have been included in the drawings of the earliest studies (Zelinka 1928) and also reported in some kinorhynch species (Zelinka 1928; Kristensen and Higgins 1991; Nebelsick 1993) (Fig.1.1).

The digestive system in kinorhynchs is a straight tube with a terminal mouth an anus divided into a foregut, midgut and hindgut (Kristensen and Higgins 1991; Neuhaus 1994; Neuhaus and Higgins 2002; Neuhaus 2013). The foregut is cuticularized and includes the mouth cone, pharyngeal crown, pharyngeal bulb and esophagus. The midgut is non-ciliated with abundant microvilli whereas the hindgut is again cuticularized and opens terminally with a rectum in segment 11 (Kristensen and Higgins 1991; Neuhaus 1994; Neuhaus and Higgins 2002).

The excretory system is composed of a single bilateral pair of protonephridia located dorsolaterally to the gut in trunk segments 8-9 (Kristensen and Higgins 1991; Neuhaus and Higgins 2002). The nephridiopore can open into a perforated cuticular area on segment 9 called sieve plate. The sieve plate varies in shape and size as well as in number of perforations among genera and species, being utilized as a taxonomic character. Moreover, it is speculated whether the protonephridia has an osmoregulatory function or not (Neuhaus 1988; Kristensen and Hay-Schmidt 1989).

Kinorhynchs have separated sexes which can usually be differentiated by the presence of dimorphic cuticular characters. Males can show pairs of penile spines or modified acicular spines. Most homalorhagid males have a pair of big ventromedial tubules on segment 2. Cyclorhagid females show thickened gonopores

or an additional pair of spines on segment 11 named lateral terminal accessory spines (LTAS). The gonads are paired and sac-shaped. Each gonad extends from segments 2-3 reaching segment 10, where they connect with the exterior through a gonopore located ventrally in between segments 10-11 (Kristensen and Higgins 1991; Neuhaus and Higgins 2002). Females show seminal receptacles in the gonoducts and thus the fertilization is assumed to be internal (Brown 1983) however, copulation has only been reported twice, once in a homalorhagid and once in a cyclorhagid species (Neuhaus and Higgins 2002; Thormar 2010). Regarding the presence of full seminal receptacles in most of the adult females of different species found (personal observation) it is most likely that a female only needs to be inseminated once or a few times to receive sperm for its entire life time.

The embryology of kinorhynchs has been studied only once in a cyclorhagid genus by Kozloff (1972, 2007). Post-embryonic development have been studied in several species showing a series of five to six moults, which gradually transform the juvenile into an adult (Neuhaus 1995; Sørensen et al. 2010a). However, some studies report molting of adults of the cyclorhagid genera *Antygomonas*, *Centroderes* and *Zelinkaderes* (Higgins 1990; Bauer-Nebelsick 1996; Neuhaus 2013).

1.4. Systematic account

From the beginning of the studies on Kinorhyncha many different classifications have been proposed mainly based in morphological characters. Currently, investigations based on combined molecular and morphological data are being carried out to propose a more accurate organization of kinorhynchs. Until then we will use the existing accepted classification, established by Zelinka (1896) and modified by Higgins in 1990, which divides the phylum into two orders: Cyclorhagida and Homalorhagida (Fig. 1.7). As reported by Neuhaus (2013) the actual classification of Kinorhyncha suffers from superordinate categories such as suborders that do not reflect the evolution in the phylum and therefore will be omitted herein.

To date the phylum Kinorhyncha comprises around 210 species (based on descriptions of adults) nested in 22 genera, that will shortly become 23 with the inclusion of a new Homalorhagid genus from abyssal depths (Sánchez et al. 2014). During the last 10 years the number of species and genera described has significantly

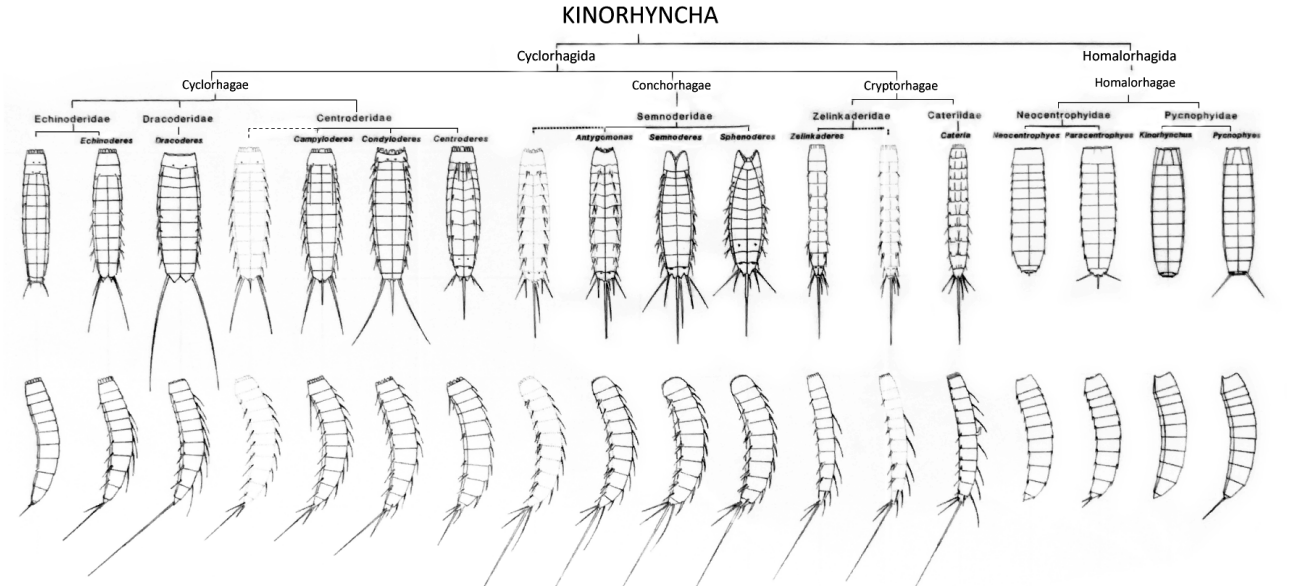


Fig. 1.7. Traditional classification of Kinorhyncha into two orders: Cyclorhagida and Homalorhagida and four suborders: Cyclorhagae, Conchorhagae, Cryptorhagae and Homalorhagae. This classification predicts the appearance of new genera within the families Centroderidae, Zelinkaderidae and Semnoderidae, depicted with dotted lines. Kindly provided by R.P. Higgins.

increased meaning that a great part of the biodiversity of the phylum is still unknown (Dal Zotto et al. 2013).

The following list, modified from Sørensen (2013), presents all the families, genera and number of species of the existing Kinorhyncha. These data include a new genus and 9 species described as a result of the present Thesis (Chapters II, III, IV, V).

Phylum KINORHYNCHA Dujardin, 1851

Order Cyclorhagida Zelinka, 1896 (7 families)

Family Antygomonidae Adrianov and Malakhov, 1994 (1 genus)

Genus *Antygomonas* Nebelsick, 1990 (4 species)

Family Cateriidae Gerlach, 1956 (1 genus)

Genus *Cateria* Gerlach, 1956 (2 species)

Family Centroderidae Zelinka, 1896 (3 genera)

Genus *Campyloderes* Zelinka, 1913 (2 species)

Genus *Centroderes* Zelinka, 1907 (1 species, soon 5)

Genus *Condyloderes* Higgins, 1969 (5 species)

Family Echinoderidae Bütschli, 1876 (5 genera)

Genus *Cephalorhyncha* Adrianov, 1999 (3 species)

Genus *Echinoderes* Claparède, 1863 (81 species)

Genus *Fissuroderes* Neuhaus and Blasche, 2006 (6 species)

Genus *Meristoderes* Herranz, Thormar, Benito, Sánchez and Pardos, 2012 (7 species)

Genus *Polacanthoderes* Sørensen, 2008 (1 species)

Family Dracoderidae Higgins and Shirayama, 1990 (1 genus)

Genus *Dracoderes* Higgins and Shirayama, 1990 (4 species)

Family Semnoderidae Remane, 1936 (2 genera)

Genus *Semnoderes* Zelinka, 1907 (3 species)

Genus *Sphenoderes* Higgins, 1969 (2 species)

Family Zelinkaderidae Higgins, 1990 (2 genera)

Genus *Triodontoderes* Sørensen and Rho, 2009 (1 species)

Genus *Zelinkaderes* Higgins, 1990 (4 species)

Family *incertae sedis*

Genus *Tubulideres* Sørensen, Heiner, Ziemer and Neuhaus, 2007 (1 species)

Genus *Wollunquaderes* Sørensen and Thormar, 2010 (1 species)

Order Homalorhagida Zelinka, 1896 (2 families)

Family Neocentrophyidae Higgins, 1969 (2 genera)

Genus *Neocentrophyes* Higgins, 1969 (2 species)

Genus *Mixtophyes* Sánchez et al., 2014 (1species)

Genus *Paracentrophyes* Higgins, 1983 (3 species)

Family Pycnophyidae Zelinka, 1896 (2 genera)

Genus *Kinorhynchus* Sheremetevskij, 1974 (19 species)

Genus *Pycnophyes* Zelinka, 1907 (53 species)

Order *incertae sedis*

Family *incertae sedis*

Genus *Franciscideres* Dal Zotto, De Domenico, Garaffoni and Sørensen, 2013 (1 species)

Cyclorhagid kinorhynchs

The present Thesis is focused on the study of the cyclorhagid kinorhynchs, one of the two extant orders. Cyclorhagida is the most widely distributed and diverse order within Kinorhyncha accommodating 17 genera and 130 species so far. Almost two thirds (81) of the total number of species belong to the genus *Echinoderes*. The remaining third is nested within 16 genera composed of one to five species each. Recent phylogenetic studies based on molecular sequences could not confirm the monophyly of the Cyclorhagida (Dal Zotto et al. 2013; Yamasaki et al. 2013). However, we still need an improved molecular taxon sampling to get a clearer idea about kinorhynch relationships.

Diagnostic characters for Cyclorhagid kinorhynchs

The order Cyclorhagida Zelinka, 1896 is characterized by the presence of a neck composed of 14-16 placids, a ring-shaped first trunk segment, which is round to oval in cross section, and cuticular spines along the trunk.

The characters with major taxonomical importance at genus level are: the number of placids in the neck and their connection with segment 1, shape of the first trunk segment, composition of second trunk segment and position and type of cuticular appendages (spines, tubules). Moreover, to discriminate at species level it is necessary to map the position and type of sensory spots and glandular openings. Additional useful characters are the pectinated fringes and cuticular hairs and adult body proportions. The distribution of cuticular characters with taxonomical relevance is often crucial to describe and discriminate species. Hence, the precise location on the body of these structures needs the establishment of a standard terminology for positions, settled for Cyclorhagida by Pardos et al. (1998) and subsequently followed by most authors (summarized in Sørensen and Pardos 2008; Neuhaus 2013).

The placids can be fused or articulated with the first trunk segment. Fused placids are present in the genera *Triodontoderes* and *Zelinkaderes* whereas articulated placids are more common varying in number and shape in remaining genera (Fig. 1.6. A-D). *Cateria* is the only cyclorhagid genus without differentiated placids (see Neuhaus 2013).

Regarding the first trunk segment, the anterior margin can be straight (in families Centroderidae, Dracoderidae, Echinoderidae and the genus Wollunquaderes), with middorsal and midventral incisions (*Antygomonas*) or showing a clam-shell like closing mechanism (*Semnoderes* and *Sphenoderes*) (Fig. 1.6. A-D).

The second trunk segment can be ring-like shaped (*Echinoderes*), composed of one tergal and two sternal

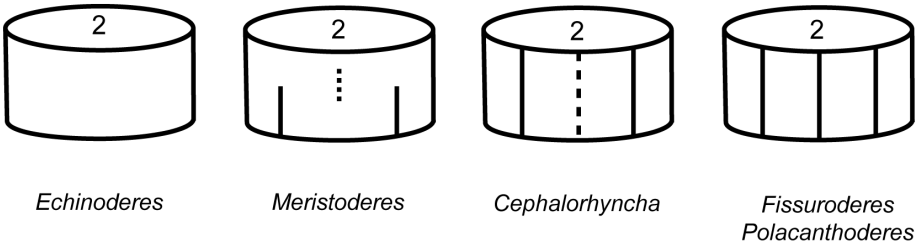


Fig. 1.8. Schematic showing the different compositions of segment 2 in cyclorhagid kinorhynchs. Dotted lines represent internal cuticular divisions that appear indistinct externally. Note the incomplete divisions in *Meristoderes*.

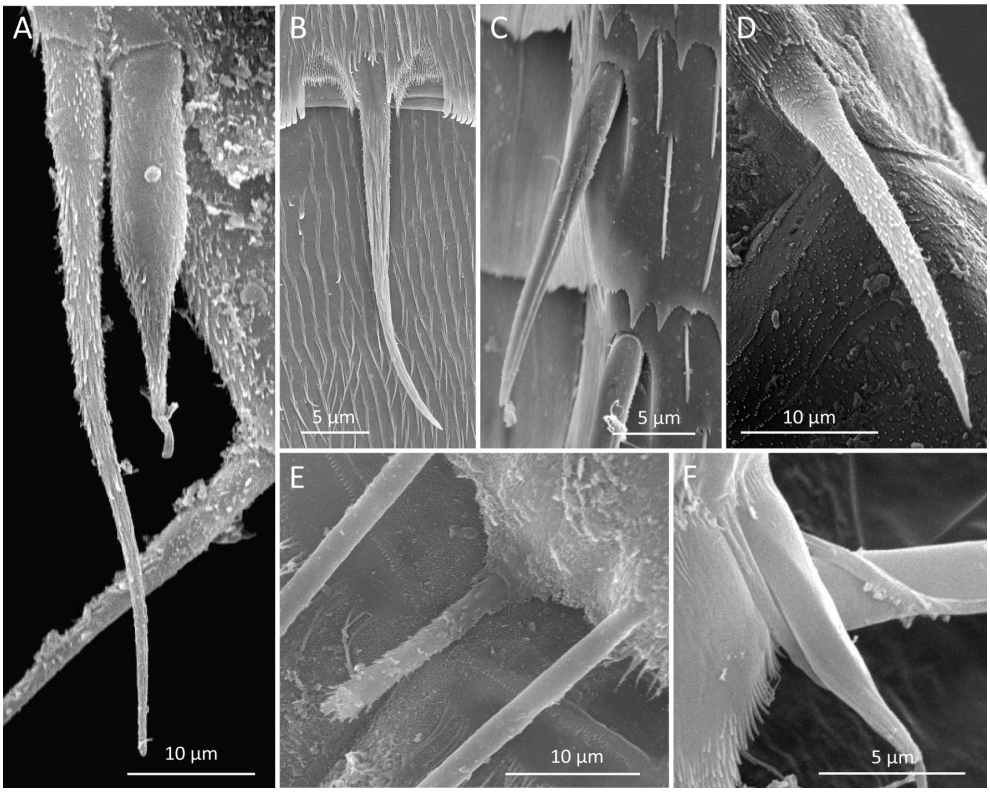


Fig. 1.9. Cuticular spines of cyclorhagid kinorhynchs, SEM micrographs. (A) *Semnoderes armiger*, acicular spine (left) and cuspidate spine (right) in lateroventral and ventrolateral positions respectively. (B) *Zelinkaderes floridensis,* middorsal acicular spine. (C) *Dracoderes abei,* lateroventral spine. (D) *Centroderes* sp. lateroventral spine. (E) *Centroderes spinosus* midventral spine-like structure. (F) *Meristoderes macracanthus,* penile spines.

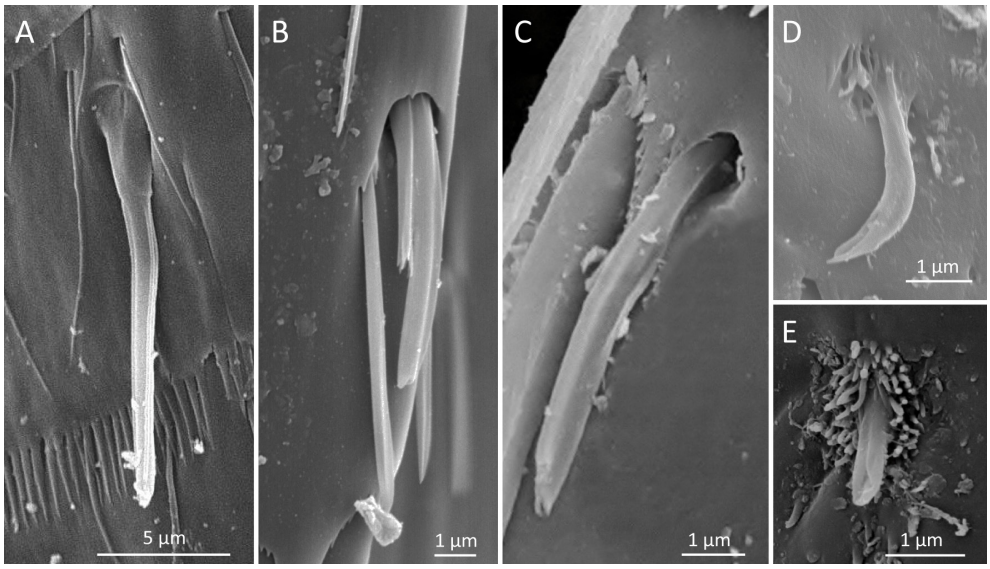


Fig. 1.10. Tubules of cyclorhagid kinorhynchs, SEM micrographs. (A) *Meristoderes macracanthus*, ventrolateral tubule. Notice division into a short basal part, and a longer distal part with wing-like extensions on the sides. (B) *Dracoderes abei*, short soft lateroventral tubule. (C) *Dracoderes gallaicus*, soft lateral accessory tubule. (D) *Meristoderes macracanthus* fringed dorsolateral tubule of females. (E) *Antygomonas* sp. ventromedial papillae.

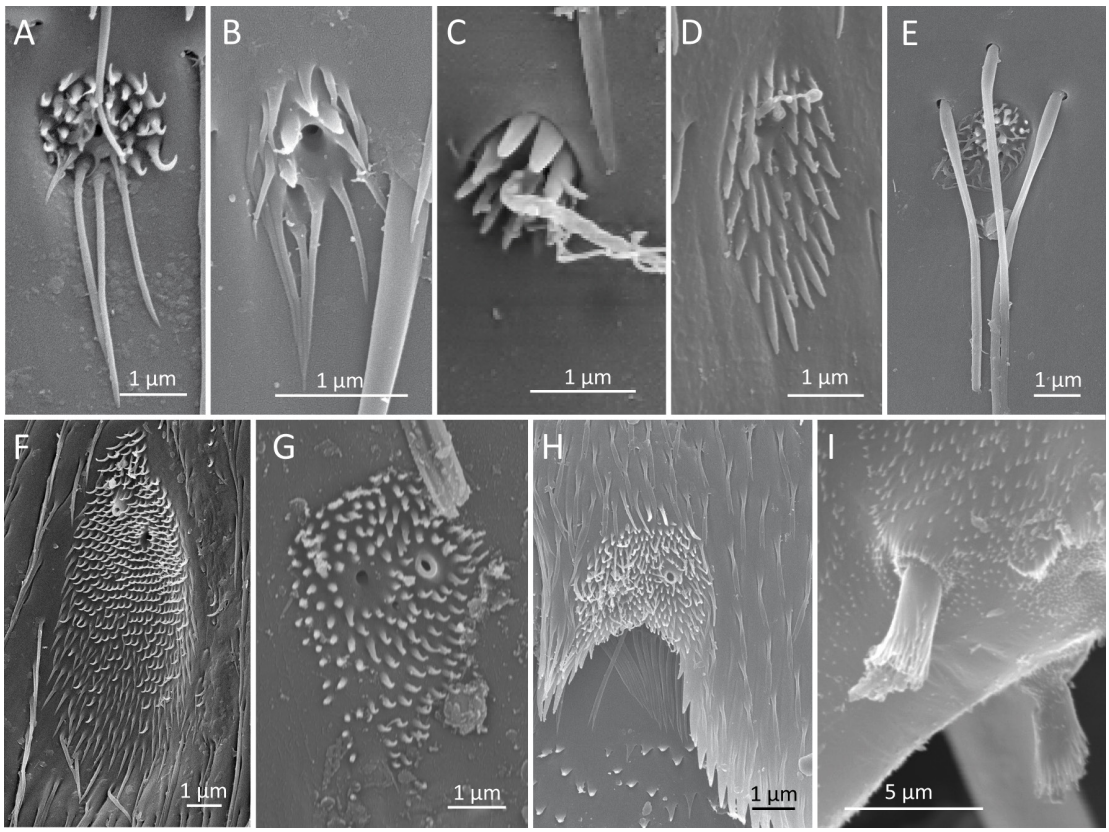


Fig. 1.11. Sensory spots of cyclorhagid kinorhynchs, SEM micrographs. (A) *Meristoderes boylei*, round sensory spot type 1 with two pores, note the three long papillae protruding. (B) *Echinoderes horni*, sensory spot type 1 with two pores and long papillae. (C) *Dracoderes gallaicus*, round sensory spot type 1 with a cilium and short papillae surrounding it. (D) *Meristoderes macracanthus* oval sensory spot type 1 with two pores and short papillae. (E) *Echinoderes horni*, round sensory spot type 1 with three associated hairs. (F) *Zelinkaderes floridensis*, oval sensory spot type 2, with two pores one of them in an elevated tube. (G) *Antygomonas paulae*, rounded sensory spot type 2. (H) *Tubulideres seminoli*, paradorsal sensory spot type 2. (I) *Antygomonas* sp. sensory spot type 3 with a single pore from an elevated tube.

plates (as in most other genera), one tergal and one sternal plate (*Cephalorhyncha*) or showing an intermediate stage in between the previous (*Meristoderes*). This character is crucial to identify the genera of the family Echinoderidae (Fig. 1.8).

The cuticular spines can be acicular or cuspidate (Fig. 1.9). The presence of acicular spines is shared by all the cyclorhagids whereas cuspidate spines are only present in the genera *Antygomonas*, *Semnoderes*, *Sphenoderes, Wollunquaderes* and *Zelinkaderes* (Fig. 1.9. A). Acicular spines can be placed in different parts of the trunk (Fig. 1.9. A-D). The most prominent are the ones positioned on segment 11 such as midterminal spine, lateral terminal spines and lateral terminal accessory spines. Moreover, the numbers and distribution of middorsal and ventrolateral spines play an important taxonomic role.

The tubules show different shapes and lengths among genera, they can be composed of a basal part and a distal end piece (Fig. 1.10. A) or be a single or multiple soft pieces (Fig. 1.10. B-E). Their arrangement is also relevant at species level.

The sensory spots exhibit a broad range of variations in size, shape and structure (Fig. 1.11) Regarding their structure they can be classified into three types: type 1, type 2 and type 3 (Nebelsick 1992a). The type 1 is the most common, showing one or two pores opening at the same level (Fig. 1.11. A-E). The type 2 show one of the two pores slightly elevated forming a cuticular tube (Fig. 1.11. F-H) and the third type appears as a

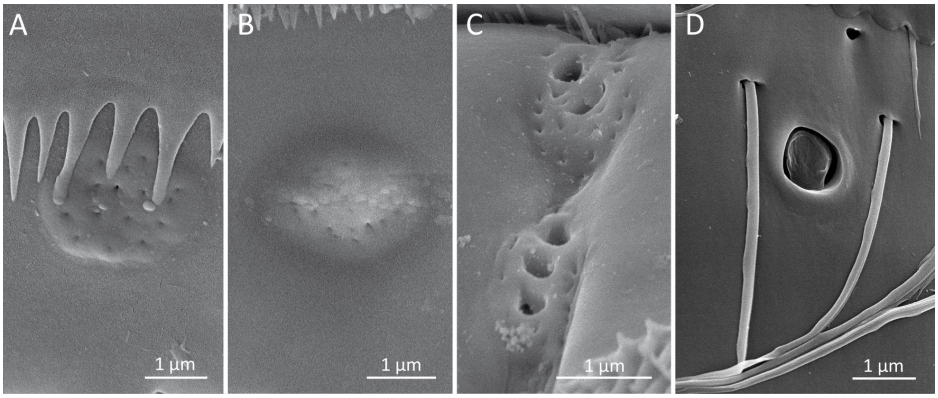


Fig. 1.12. Glandular outlets of cyclorhagid kinorhynchs, SEM micrographs. (A) *Echinoderes horni*, glandular cell outlet type 1 (pore field). (B) *Echinoderes spinifurca*, glandular cell outlet type 1. (C) *Fissuroderes sorenseni*, middorsal glandular cell outlets type 1 on segment 11. (D) *Echinoderes horni*, glandular cell outlet type 2 (single opening gland outlet).

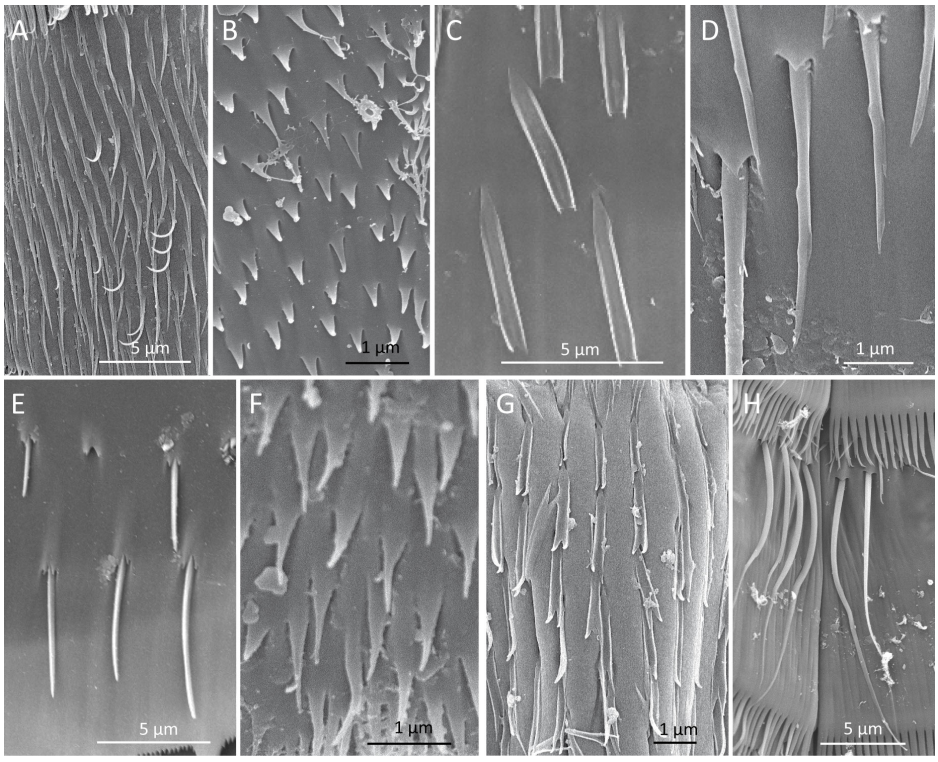


Fig. 1.13. Cuticular hairs of cyclorhagid kinorhynchs, SEM micrographs. (A) *Zelinkaderes floridensis,* aligned hairs without perforation sites (B) *Tubulideres seminoli*, short scale-like cuticular hairs without perforation sites. (C) *Antygomonas paulae*, leaf-like cuticular hairs. (D) *Echinoderes spinifurca*, bracteated hairs. (E) *Dracoderes gallaicus*, double bracteated hairs. (F) *Centroderes* sp., short scale-like cuticular hairs without perforation sites. (G) *Zelinkaderes brightae*, cuticular hairs without perforation sites, bifurcated distally and arranged in rows. (H) *Meristoderes macracanthus*, paraventral patches of long bracteated hairs

protruding cone surrounded by papillae and a single pore (Fig. 1.11. I).

The gland cell outlets can be categorized into two types: type 1 or pore field and type 2 or funnel-shaped. The first type has numerous pores (Fig. 1.12. A-C) while the second type just shows a big single pore opening surrounded by a funnel-shaped subcuticular structure (Fig. 1.12. D).

Cuticular hairs show a great morphological variation: filiform, leaf-like, scale-like, etc. (Fig. 1.13). Moreover the hairs can emerge from perforations of the cuticle (herein named perforation sites) or not. The use of cuticular hairs in cyclorhagid taxonomy varies among species, since some look very much alike whereas

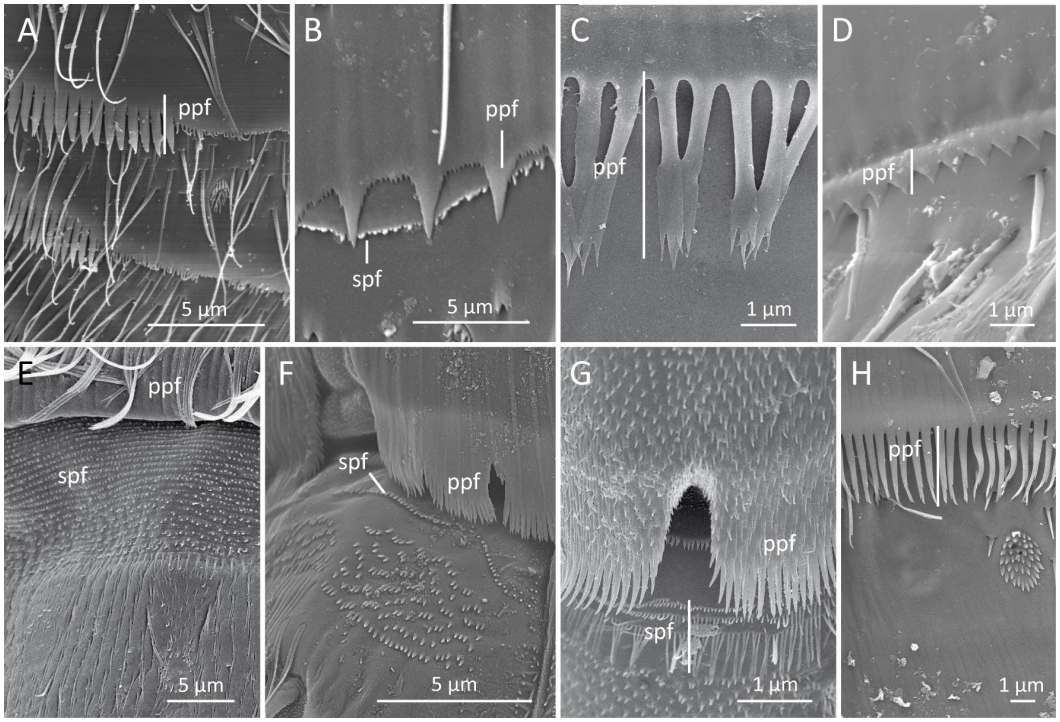


Fig. 1.14. Pectinate fringes of cyclorhagid kinorhynchs. (A) *Echinoderes worthingi,* primary pectinate fringe of first and second segments showing a transition from well-developed fringe tips to minute and flexible ones. (B) *Dracoderes gallaicus*, serrated primary pectinate fringe, minute secondary pectinate fringe. (C) *Meristoderes boylei,* trident-shaped primary pectinate fringe. (D) *Meristoderes macracanthus,* minute primary pectinate fringe from segment 1 with triangular fringe tips. (E) *Zelinkaderes floridensis,* primary pectinate fringe with soft fringe tips, that appear bent, multiple minute secondary pectinated fringes. (F) *Antygomonas paulae*, short primary pectinate fringe, one secondary pectinate fringe forming a wavy line. (G) *Tubulideres seminoli,* long and rounded primary pectinate fringe, with three secondary pectinate fringes increasing posteriorly in length. (H) *Meristoderes macracanthus,* long and flexible pectinate fringe. Abbreviations: ppf, primary pectinate fringe; spf, secondary pectinate fringe.

others are very distinct and form conspicuous patterns.

Pectinate fringes are present at many posterior cuticular margins of segments. The size and shape of these fringes varies greatly among species and genera (Fig. 1.14). Other fringes, namely secondary fringes, appear in the anteriormost part of the segments usually covered by the free end of the preceding segment (Fig. 1.14. B, E-G).

1.5. Phylogenetic frame

The position of poorly known, sometimes called “minor” phyla such as Kinorhyncha seems to hold key positions in metazoan phylogeny, linking various invertebrate lines into new units and therefore has always been objet of interest (Giere 2009). Since the creation of the polyphyletic group Aschelminthes also named Pseudocelomates or Nemathelminthes (Lang 1963; Nielsen 1995; Schmidt-Rhaesa 2007), where kinorhynchs were grouped, the position of the phyla has changed. The improvement of the molecular techniques and increase

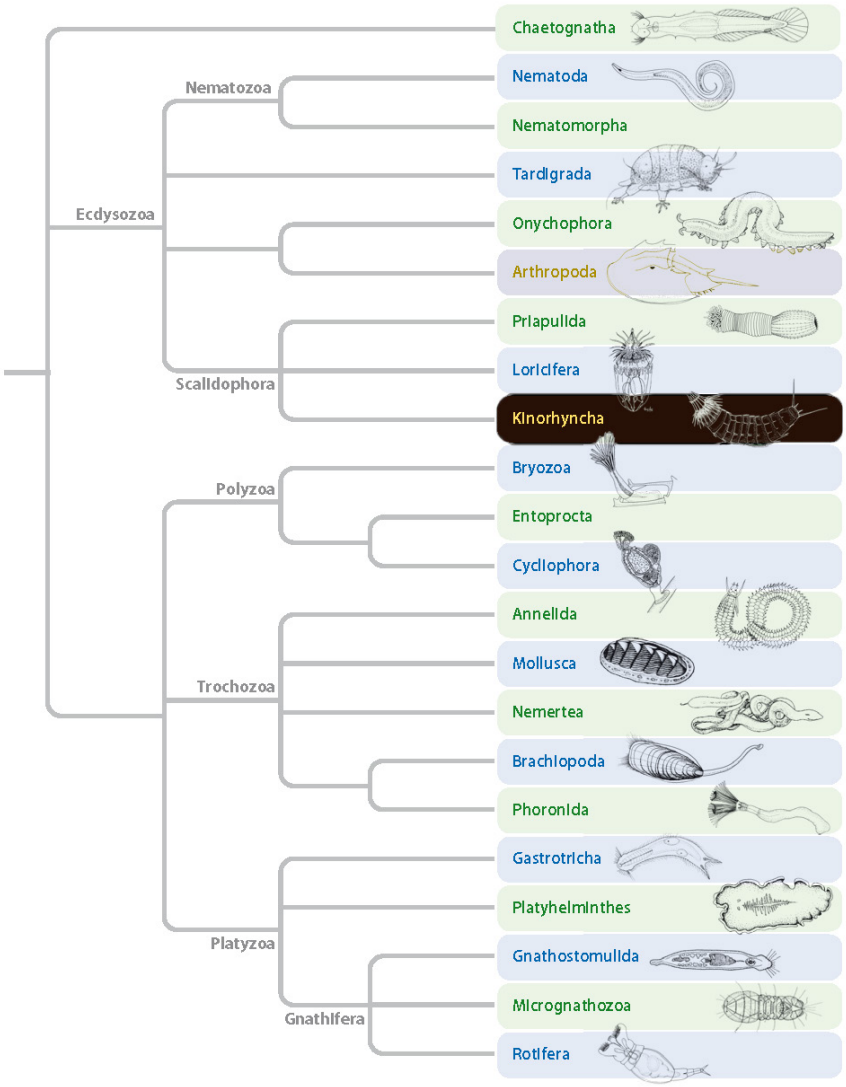


Fig. 1.15. Hypothesis of the protostome tree of life. This tree is a summary from diverse sources which emphasizes groups recognized in phylogenetic analyses. Kinorhynchs are marked in black. Modified from Giribet and Edgecombe (2012).

of the studies focused in these minor phyla allowed to relocate the multiple taxa previously assigned to the Aschelminthes as part of other groups. Kinorhynchs were positioned within the Ecdysozoa, one of the two protostome monophyletic superclades (Aguinaldo et al. 1997) including arthropods, tardigrades, onychophorans, nematodes, nematomorphs, loriciferans and priapulids (Mallatt et al. 2004; Schmidt-Rhaesa et al. 1998; Telford et al. 2008; Edgecombe et al 2011) (Fig. 1.15). The defining apomorphy of this clade is the periodic molting of the cuticle as the animal grows (a process known as ecdysis) and the absence of a ciliated epithelium (Aguinaldo et al. 1997; Edgecombe et al 2011; Nielsen 2012). Within Ecdysozoa, kinorhynchs are allied to nematomorphs, nematodes, priapulids and loriciferans in the clade Cycloneuralia (Schmidt-Rhaesa 1998; Schmidt-Rhaesa 2007; Nielsen 2012) (Fig. 1.16). The name Cycloneuralia refers to the presence of an "apomorphic" collar-shaped brain surrounding the pharynx (Ahlrichs 1995), however the monophyletic status of the group is still under debate (Garey 2001; Telford et al. 2008; Budd and Telford 2009; Edgecombe et al. 2011). Morphologists have also united priapulids, kinorhynchs and loriciferans as the Scalidophora (Lemburg

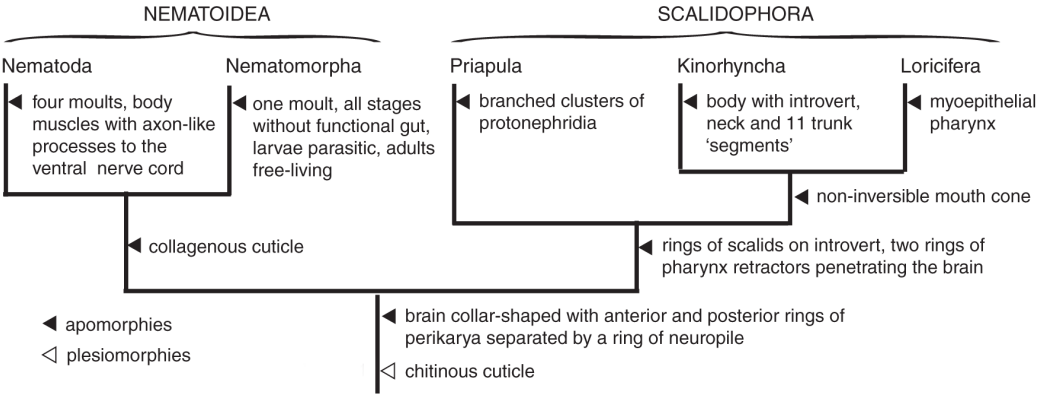


Fig. 1.16. Phylogeny of the Cycloneuralia including apomorphies and plesiomorphies for each group, from Nielsen (2012).

1995; Schmidt-Rhaesa 1998) or Cephalorhyncha (Nielsen 2001), on the basis of a shared introvert with scalids and the presence of two rings of retractor muscles on the introvert (Telford et al. 2008) (Figs 1.15, 1.16). Most of the phylogenetic studies place kinorhynchs together with priapulids as sister groups both considered to occupy a basal position within Ecdysozoa (Mallatt et al. 2004; Mallatt and Giribet 2006; Giribet et al. 2007; Dunn et al. 2008; Hejnol et al. 2009). The phylogenetic position of loriciferans, not included in the mentioned studies and most likely sister group of either priapulids or kinorhynchs, is still far from settled (Park et al. 2006; Sørensen et al. 2008). Consequently, the exact branching pattern among the three groups is still under debate (Schmidt-Rhaesa 2007).

The phylogeny within Kinorhyncha is far to be resolved (Neuhaus 2013). Over the course of the years, numerous studies have somehow discussed evolutionary scenarios based on morphological characters, most of them in the frame of taxonomic and morphologic descriptions (Lang 1949; Adrianov and Malakhov 1994; Neuhaus 1993, 1994, 1995; Neuhaus et al. 2013; Sørensen and Rho 2009; Sørensen and Thormar 2010; Neuhaus and Sørensen 2012). An attempt to resolve relationships at lower taxonomic level was carried out by Gª Ordóñez et al. (2008) in the genus *Echinoderes* combining several morphological characters in an evolutionary context but not based on rigorous cladistic methodology. The first attempt to combine modern numerical methods with morphological characters under a cladistic approach was performed by Sørensen (2008b) however, this study was focused just in the family Echinoderidae. More recently, for the first time, a couple of molecular phylogenetic studies have been carried out addressing general inter-relationships within the phylum (Yamasaki et al. 2013; Dal Zotto et al. 2014). Both studies roughly corroborate the actual classification pointing out the misplacement of the genus *Dracoderes* within Cyclorhagida. Following the results of these studies *Dracoderes* should be placed within Homalorhagida. Additionally, a taxonomic study on species of *Dracoderes* included in the present Thesis (Chapter III) shows suggestive shared morphological traits among this genus and Homalorhagid genera. However, further phylogenetic studies with a broader taxon sampling are still needed to resolve the uncertainties of the present studies.

1.6. History of the studies on Kinorhyncha and state of the art

In 1851, ten years after discovering the first kinorhynch in Saint Malo, the French scientist Felix Dujardin published the first study entitled "*Sur un petit animal marin, l'Échinodère, formant un type intermédiaire entre les Crustacés et les Vers*". However, the first description of a species was not published until 1863 when

Claparède, another French zoologist, described *Echinoderes dujardinii* (Claparède 1863). During the same period (1863-1868), kinorhynchs were mentioned in several studies but the first extensive work was carried out by Greeff in 1869 who also described a new species from the Canary Islands. Curiously, since the discovery of the phylum all the investigations and reports referred to cyclorhagids until the studies of Reinhard (1881, 1887) which included the description of the first homalorhagids. Reinhard was also the responsible for the current name of the phylum, proposed as an alternative to the name Echinodera as explained in the first part of the introduction of this Thesis.

From 1841-1920, almost 70 years after the discovery of the phylum, only a few kinorhynch species were formally described. This situation changed dramatically with the publication of Karl Zelinka's monograph (1928) entitled ''Monographie der Echinodera'' becoming one of the greatest contributions in kinorhynch science. This monograph included a complete revision of the systematic system of Reinhard (1881, 1887), providing for the first time aspects of the biology of kinorhynchs and describing 48 species of which 12 still remain valid (Neuhaus 2013). Despite the fact that Zelinka was using a simple, monocular microscope, he was able to see details that have just recently been confirmed by modern Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM).

From 1930-1960 there were numerous individual contributions published by several authors (e.g. Blake 1930; Lang 1936, 1949, 1953; McIntyre 1962) but a major progress in kinorhynch taxonomy was accomplished by the studies of Robert P. Higgins and co-workers after 1960 (Neuhaus 2013). They described a total of 6 genera, 60 new species and redescribed 14 species (e.g. Higgins 1960, 1966, 1967, 1968, 1969a-b, 1982, 1983, 1990; Brown and Higgins 1983; Higgins and Kristensen 1988; Higgins and Shirayama 1990; Higgins and Adrianov 1991; Pardos et al. 1998; Martorelli and Higgins 2004).

Besides Higgins, a small group of scientists, most of them Higgins' students and co-workers, have been contributing to kinorhynch taxonomy and diversity for more than 15 years. It is remarkable the work of MV. Sørensen with 33 new species and six new genera; A. Adrianov with 26 new species and one new genus; B. Neuhaus with six new species and two new genera and our own research group with 25 new species and two new genera (e.g. Adrianov 1989, 1995; Pardos et al. 1998; Adrianov and Malakhov 1999; Sørensen et al. 2000, 2005, 2007, 2010b-c, 2012; Neuhaus 2004; Neuhaus and Blasche 2006; Sørensen 2006, 2008a,b; Gª Ordóñez et al. 2008; Sørensen and Thormar 2010; Thormar and Sørensen 2010; Sánchez et al. 2011; Herranz et al. 2012; Neuhaus and Sørensen 2012; Neuhaus et al. 2013). Nowadays the best investigated areas in the world are the Mediterranean Sea, the East coast of North America and the recently surveyed coasts of Korea and the Iberian Peninsula (see Chapter I).

Along the last 40 years, other investigations have been carried out apart from taxonomy. The beginning of the use of the transmission electron microscopy (TEM) in kinorhynchs lead to a better understanding of the general internal morphology of the phylum (Kristensen and Higgins 1991; Neuhaus 1991, 1994; Adrianov and Malakhov 1994). Posteriorly, more detailed TEM studies focused in single organs such as nephridia, gut, sensory organs, nervous system, etc. allowed a deeper insight into the functional morphology of the phylum (Neuhaus 1988, 1991, 1994, 1997; Kristensen and Hay-Schmidt 1989; Nebelsick 1992a, b; Nebelsick 1993). The use of the SEM for taxonomic purposes offered a higher optical resolution of surface structures, allowing better and more detailed descriptions of cuticular characters, which could not be studied in depth with only LM such as the introvert arrangement, sensory spots, spines, tubes, etc. The use of SEM combined with LM improved the accuracy of descriptions providing a whole view of the variability of cuticular structures within the phylum. The first species that were described only using SEM were *Echinoderes hispanicus* and *Echino-*

deres cantabricus (Pardos et al. 1998).

Another important advance in kinorhynch morphology is expected from the introduction of new technical approaches such as the confocal laser scanning microscopy. The only and first studies until the papers presented herein were performed on the muscle system by labeling of F-actin with Phalloidin in two different genera: *Antygomonas* and *Pycnophyes* (see Müller and Schmidt-Rhaesa 2003; Rothe and Schmidt-Rhaesa 2004; Schmidt-Rhaesa and Rothe 2006).

Developmental studies were initiated by Remane (1936) who discovered that kinorhynch development is direct and without a metamorphosis. Nyholm (1947) wrote the first information about a kinorhynch life history obtained by culturing *Echinoderes*, posteriorly Kozloff (1972, 2007) improved Nyholm's results and documented nine-segmented juveniles of *Echinoderes* hatching from the egg. Additional studies in the post-embryonic development have been performed in different taxa by several authors including Higgins (1974), Brown (1985), Neuhaus (1995) and Sørensen et al. (2010a) among others.

Current studies are focused both in the study of unexplored areas to describe new taxa and in the study of kinorhynch morphology trough modern techniques such as the CLSM studies included in the present Thesis (Chapters VI, VII, Appendix II). On the other hand phylogenetical studies are starting to include molecular data (Yamasaki 2013; Dal Zotto 2013). However, as reported above (see phylogenetic frame section), these studies need to be improved to get a better resolution to resolve with more detail the relationships among the phyla. This is currently being accomplished thanks to a joined effort of several scientists, a task in which our research group is involved. Unfortunately, nowadays embryologic and developmental studies are not being successful due to the difficulties presented in the culturing of Kinorhynchs.

Studies in Spain

The studies of Kinorhynchs in Spanish waters have always been scarce being reduced to isolated recordings from ecological investigations (e. g, Villora 1993; Arroyo 2002). The first described kinorhynch in Spain was *Echinoderes canariensis* from Greeff (1869) reported from Lanzarote and qualified as *species inquirendum* (Pardos et al. 1998) but recently considered a valid species (Neuhaus 2013). Additionally several authors reported *Echinoderes* from Mallorca coasts namely *E. sieboldii* Pagenstecher, 1875, *E. incertus* Reinhard, 1885 and *E. pagenstecheri* Reinhard, 1885 which later was synonymized with *E. dujardinii* (Pardos et al. 1998). Since then, there were no records for more than one century until Pardos, Higgins and Benito (1998) described two new species of kinorhynchs from the north coasts of the Iberian Peninsula: *Echinoderes hispanicus* and *Echinoderes cantabricus*. Posteriorly, F. Pardos and J. Benito leaded a series of research projects funded by the Spanish Government and focused on the study of Kinorhyncha in an extensive coastal area around the Iberian Peninsula. These studies, still active, began with the North Coast followed by the North West, South and East coasts of Spain. Three new species were described from the North coast study: *Echinoderes isabelae*, *Echinoderes parrai* and *Echinoderes neospinosus* (Gª Ordóñez et al. 2008). In addition to these five species, there are many new reports and description of new taxa as a result of our current research activity summarized in the biogeographical part of this Thesis (Chapter I and Appendix I). The present thesis, partially framed in the long-term project mentioned, includes a biogeographical study of the cyclorhagid kinorhynchs of the Iberian Peninsula (Chapter I), two publications of new taxa (Chapters II, III) and additional unpublished results of ongoing studies in the area (Appendix I).

1.7. Current problems on the study of Kinorhynchs

Even though Kinorhyncha is a very interesting meiofaunal group there is a small active research community working on them (less than 10 specialists around the world). General studies in meiofauna rarely report them due to their patchy distribution, tiny size and difficulty of their separation from fine muddy sediments. Additionally, when they are reported is usually as Kinorhyncha ssp. or simply among "other groups". Such reports result in the loss of a lot of valuable information. Moreover, cyclorhagid kinorhynchs can be easily confused with copepods and missed in the meiofaunal collections. As a result, many of the global areas still remain unexplored or underexplored for kinorhynchs and specific studies need to be carried out.

Despite the fact that cyclorhagids are the most reported kinorhynchs, the knowledge of their organ systems is still very limited maybe due to their general small size. To date, most of the studies on the morphology in kinorhynchs describe detailed ultrastructure of particular regions such as the head, or sensory organs based on TEM and generally dealing with no more than tree genera. The presence of the cuticle is a handicap to study the organ systems through specific cytochemical and immunocytochemical experiments. Similar problems occur with molecular techniques where a single specimen is sometimes not enough to extract a suitable amount of DNA. Fortunately, the improvement of the techniques and the adjusting of protocols are making easier the labor of our small community.

Regarding the taxonomy, with the course of the years and the improvement of microscopic techniques, the taxonomic important characters and terminology for cyclorhagid kinorhynch have been modified and new terms have been included. Unfortunately, this terminology has not always followed a strict consensus in all the studies and descriptions, and therefore need a more standardized protocol. Some of the last review publications such as Sørensen and Pardos (2008) and Neuhaus (2013) have been establishing a common terminology that is being followed and completed in upcoming studies. Current usage and definitions are summarized in Neuhaus (2013).

1.8. Objectives

In the context of the previous introduction it seems clear that the studies of Kinorhynchs are still in an early stage. There is a substantial lack of knowledge in many areas of study such as taxonomy, biogeography, ecology, morphology, whereas others are just starting to be explored, for instance the molecular phylogeny and the developmental biology. The selected group of study is the order Cyclorhagida, showing a worldwide distribution and nearly 130 species and 18 genera, but comprising the smallest sized species.

The general objective of this Thesis is to contribute to integrate three different approaches (biogeographical, taxonomical and morphological) to increase the current knowledge of the cyclorhagid kinorhynchs with a broad perspective.

Biogeography

Despite the wide distribution of cyclorhagid kinorhynchs, our current information about their concrete geographical distribution is extremely fragmented and merely a reflection of the activities of the few experts that have studied the group through time.

The Iberian Peninsula was almost an unexplored area until the late 1990s when our research group initiated a series of projects funded by the Spanish government. This thesis was initiated in the frame of these studies including additional areas where we got the chance to collect such as Florida East coast, Naples (Italy)

and the Atlantic coast of Panama. As a result of collaborations with other meiofauna researchers we gathered data from the South of Portugal and South of France.

The aims of the biogeographical studies were:

- To carry on for the first time an intensive sampling of the meiobenthos along the coast of the Iberian Peninsula in order to determine species composition and distribution of the cyclorhagid kinorhynchs.
- To provide a homogenized data set of all available records of cyclorhagid kinorhynchs in the Iberian Peninsula coasts.
- To compare the diversity of the cyclorhagid kinorhynch species in the Atlantic vs. Mediterranean coasts of the Iberian Peninsula.
- To identify potential associations between species and different kinds of benthic habitats, through ecological factors such as type of sediment and depth.
- To study the diversity of cyclorhagid kinorhynchs in additional areas.
- To initiate studies of cyclorhagid kinorhynch populations in historically well sampled areas to compare the present diversity with the reported before in the same area.

Taxonomy

As a result of the multiple samplings carried out in the Iberian Peninsula and additional areas several new species and one genus were discovered. In this context the main objectives were:

- To describe the new taxa found and redescribe already known species that have doubtful and incomplete descriptions.
- To improve and homogenize the established taxonomical standards for the description of new species.
- To evaluate the accuracy and relevance of extant characters as taxonomic tools and provide new characters with potential phylogenetic significance.

Morphology

The use of the confocal laser scanning microscopy added a new dimension to our understanding of fundamental organ systems and their evolution in animals. The musculature and the nervous system are the only internal and segmental organ systems in kinorhynchs, therefore seem to be important in the study of the evolution of segmentation within Ecdysozoa.

The aims of the morphological studies were:

- To study the segmented internal organ systems in cyclorhagids, (muscle system and nervous system) using CLSM in order to complement previous studies.
- To understand the function of the different sets of muscles and describe new findings.
- To initiate and standardize the application of CLSM techniques to the study of the nervous system in cyclorhagid kinorhynchs.
- To describe and compare nervous system features among different cyclorhagid genera and species integrating

and interpreting the obtained results in an evolutionary context.

The present Thesis is divided into three mayor parts according to the approaches referred to above: biogeography, taxonomy and morphology. This organization mirrors the order followed during the course of the investigation starting with the general biogeographical studies followed by the more concrete studies at genus and species level on the taxonomy and morphology.

The biogeographical part includes a single article and an Appendix with complementary data.

Kinorhyncha from the Iberian Peninsula: new data from the first intensive sampling campaigns includes a general characterization of the distribution of cyclorhagid kinorhynchs along the coasts of the Iberian Peninsula. It also provides a catalogue of the entire known cyclorhagid kinorhynchs for the area including possible associations among species, sediment type and depth.

Appendix I includes complementary information from additional sampling points along the Iberian Peninsula and Ceuta carried out after the publication of the first paper. Moreover, additional information on different areas recently sampled is provided.

The taxonomical part includes four publications:

***Meristoderes* gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae)** includes the description of a new genus with two species one from the Mediterranean coast of Spain and the other from the Solomon Islands as a result of a collaboration with Jonas Thormar, a Danish researcher and former research student of Dr. MV. Sørensen. This paper also contains an evaluation of morphological characters and their phylogenetic importance in the family Echinoderidae, the most specious within Kinorhyncha.

On the genus *Dracoderes* Higgins & Shirayama, 1990 (Kinorhyncha: Cyclorhagida) with a redescription of its type species, *D. abei*, and a description of a new species from Spain includes a revision of this interesting genus original from Japanese waters with the redescription of the type species *D. abei* and the surprising appearance of a new congeneric species in the Atlantic coast of Spain. Additionally, new morphological data are provided for the first time in the genus opening new discussions of the phylogenetic position within Kinorhyncha. This publication was also in collaboration with Danish, Japanese and Korean researchers.

New Kinorhyncha from Florida coastal waters is a complement to the studies initiated by Robert P. Higgins in the Atlantic coast of Florida. It includes the description of four new species, three of them cyclorhagids of two different genera, and a homalorhagid species.

***Fissuroderes sorenseni* sp. nov. and *Meristoderes boylei* sp. nov.: First Atlantic recording of two rare kinorhynch genera, with new identification keys** includes the description of two species belonging to two poorly known cyclorhagid genera found in the Atlantic coast of Florida including new identification keys. It also provides for the first time SEM images and a detailed description of the introvert in *Fissuroderes* revealing new characters that might have phylogenetic significance.

The morphological part includes two publications and Appendix II. The studies in this part are merely focused in two organs systems: the musculature and the nervous system both considered very interesting in an evolutionary context for their segmental nature.

Comparative myoanatomy of *Echinoderes* (Kinorhyncha, Cyclorhagida): A comprehensive investigation by CLSM and 3D reconstruction (submitted)

This publication makes a comparative study of the whole myoanatomy in five species from the most specious genus of the cyclorhagid kinorhynchs (*Echinoderes*), utilizing cytochemical labeling techniques and CLSM. It includes for the first time three-dimensional reconstructions and a complete description of the musculature of the head of a kinorhynch. Detailed functional interpretations are also provided as well as comparisons among genera and phyla in an evolutionary context.

Comparative Morphology of Serotonergic-Like Immunoreactive Elements in the Central Nervous System of Kinorhynchs (Kinorhyncha, Cyclorhagida)

This publication is a comparative study among different cyclorhagid genera using a single marker (serotonin). It represents the first successful attempt to study the nervous system through immunocytochemical techniques in the phylum and imply the beginning of a series of ongoing studies. It also provides a standardized protocol adapted to the special nature of kinorhynchs.

Appendix II includes complementary data of the previous investigation on the nervous system providing preliminary results of an ongoing research carried out in the mentioned organ system combining different markers.

The ocean's bottom is at least as important to us as the moon's behind.

Gordon G. Lill

2

General Materials and Methods

2. General materials and methods

2.1. Sampling areas

The studies of the present thesis were based on material collected from the Iberian Peninsula, Florida and Naples, and material collected in collaboration with other scientists in France, Panama, South Korea, Japan and the Solomon Islands (Fig. 2.1). Extensive systematic samplings were only carried out in the Iberian Peninsula and Naples whereas in remaining areas the samplings were more scattered.

Detailed maps and information of the sampling points are recorded in the material and methods of each publication and in Appendix I (Figs A.I. 1-5, Tables A.I. 1-4).

Fig. 2.1. Overview map showing sampling areas of this study.

2.2. Material examined

Around 1200 specimens belonging to 42 different species were studied during the course of this thesis. All examined material is summarized in Table 2.1.

Table 2.1. List of the material examined included in the publications of the present thesis and Appendix I. Numbers refer to the specimens studied with LM. Specimens used for SEM and CLSM studies, and additional not mounted specimens from each locality were not taken into account.

Species	Sampling area	Studied specimens	Chapter
Antygomonas gwenae	Florida (USA)	3	IV
Antygomonas paulae	Florida (USA)	20	VII, Appendix I
Antygomonas incomitata	Blanes (Spain)	10	I
***Antygomonas* sp.1**	Ría de Vigo, Ría de Arosa, Ceuta (Spain)	90	I
***Antygomonas* sp.2**	Ría de Vigo, Ría de Arosa (Spain)	50	Appendix I
Campyloderes* cf. *vanhoeffeni	Algeciras, Isla Grosa, Denia (Spain)	4	I
***Campyloderes* sp.**	Bocas del Toro (Panama)	1	Appendix I
Centroderes spinosus	Blanes (Spain) Banyuls (France)	15	I
***Centroderes* sp.1**	Florida (USA)	5	Appendix I
***Centroderes* sp.2**	Bocas del Toro (Panama)	15	Appendix I
***Condyloderes* sp.**	Llanes, San Vicente (Spain) Naples (Italy)	1	I
Dracoderes gallaicus	Ria de Arosa, Ría de Vigo, Algeciras Bay, Malaga (Spain) Banyuls (France), Faro (Portugal)	60	III
***Dracoderes* sp.**	Ishigaki Island (Japan)	13	III
Echinoderes adrianovi	Florida (USA)	1	IV
Echinoderes cantabricus	Santoña, Ría Ferrol, Ría de Vigo, Ría de Arosa, Cádiz, Algeciras Bay, Almuñécar, Garrucha, Isla Grosa, Málaga (Spain)	200+	I
Echinoderes dujardinii	Ría de Arosa, Cádiz, Huelva, Algeciras Bay, Almuñécar, Denia, Isla Grosa, Ceuta (Spain) Faro, Albufeira (Portugal)	100+	I
Echinoderes hispanicus	Santoña, Ría Ferrol, Ría de Vigo, Ría de Arosa, Cádiz, Algeciras Bay, Almuñécar, Garrucha, Isla Grosa, Málaga, Blanes, (Spain) Faro (Portugal)	200+	I
Echinoderes kristenseni	Ría de Arosa (Spain)	1	I
Echinoderes riceae	Florida (USA)	30	IV
Echinoderes spinifurca	Florida (USA)	20	VI, Appendix I
Echinoderes* cf. *worthingi	Ría de Ferrol, Huelva, Cádiz, Málaga (Spain)	50	I
***Echinoderes* sp.1**	Cudillero, Ría de Arosa, Almuñécar, Denia, Blanes (Spain) Faro, Albufeira (Portugal)	100+	VI, Appendix I
***Echinoderes* sp.2**	Malaga, Algeciras, Huelva, Cádiz (Spain) Albufeira (Portugal)	70	Appendix I
***Echinoderes* sp.3**	Blanes (Spain)	8	Appendix I
***Echinoderes* sp.4**	Blanes (Spain)	10	Appendix I
***Echinoderes* sp.5**	Faro (Potugal)	15	Appendix I

***Echinoderes* sp.6**	Faro (Potugal)	10	Appendix I
***Echinoderes* sp.7**	Naples (Italy)	3	Appendix I
***Echinoderes* sp.8**	Naples (Italy)	2	Appendix I
***Echinoderes* sp.9**	Naples (Italy)	3	Appendix I
***Echinoderes* sp.10**	Naples (Italy)	2	Appendix I
***Echinoderes* sp.11**	Bocas del Toro (Panama)	5	Appendix I
***Echinoderes* sp.12**	Bocas del Toro (Panama)	2	Appendix I
***Echinoderes* sp.13**	Naos (Panama)	15	Appendix I
Fissuroderes sorenseni	Florida (USA)	5	V
Meristoderes macracanthus	Blanes (Spain) Naples (Italy)	100+	II
Meristoderes galatheae	Ghizo Island (Solomon Islands)	5	II
Meristoderes boylei	Florida (USA)	4	V
Semnoderes armiger	Comillas, Llanes, Navia, Denia, Blanes (Spain) Naples (Italy)	40	I
Semnoderes* cf. *pacificus	Banyuls (France)	1	I
***Sphenoderes* sp.**	Faro (Portugal)	1	Appendix I
Tubulideres seminoli	Florida (USA)	20	Appendix I
Zelinkaderes floridensis	Florida (USA)	5	Appendix I
Zelinkaderes brightae	Florida (USA)	20	VII, Appendix I

2.3. Material loaned from collections

Apart from the material collected in the mentioned samplings, additional material from scientific collections at museums was necessary in order to compare characters among similar species (Table 2.2).

Table 2.2. List of the loaned material from scientific collections at museums

Species	Number of specimens	Museum	Acknowledgments
Cephalorhyncha liticola	2	Zoological Museum, Natural History Museum of Denmark	Martin V. Sørensen
Dracoderes abei	5	Zoological Museum, Natural History Museum of Denmark	Martin V. Sørensen
Fissuroderes higginsi	1	Museum für Naturkunde, Berlin	Birger Neuhaus
Fissuroderes novaezealandia *	2	National Institute of Water and Atmospheric research (New Zealand)	Birger Neuhaus
Fissuroderes papai	3	Museum für Naturkunde, Berlin	Birger Neuhaus
Fissuroderes rangi *	2	National Institute of Water and Atmospheric research (New Zealand)	Birger Neuhaus
Fissuroderes thermoi	4	Museum für Naturkunde, Berlin	Birger Neuhaus

* Only pictures

2.4. Sampling

All the samplings carried out were qualitative. Sediment samples were collected both intertidal and subtidally. Intertidal samples were collected at low tide using a shovel to scoop the upper portion of the sediment. Subtidal sediment samples were collected from a boat by deployment and retrieval of either a Higgins meiobenthic dredge or an anchor dredge (Fig. 2.2). Both devices are prepared to collect the uppermost (oxygenated) part of the sediment only, where the kinorhynchs are supposed to live. This way they maximize the surface sampled and minimize the amount of "empty" sediment from deeper layers.

Samples taken during the fisheries campaign CARIOCA90, conducted by the Spanish Institute of Oceanography, used a cylindrical collecting tool (15 cm diameter and 40 cm length) attached to a fisheries bottom trawl (Fig. 2.2.E).

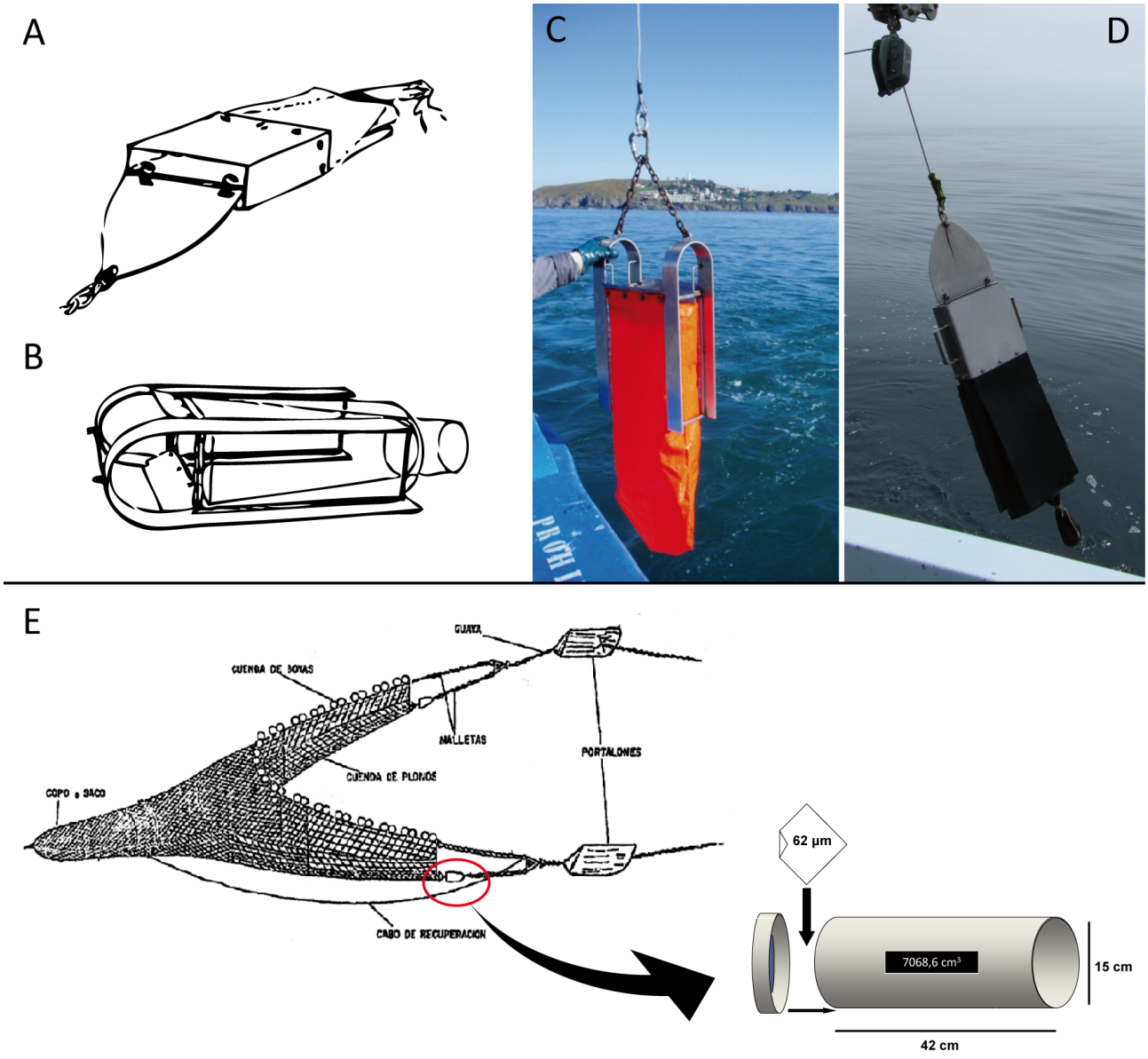


Fig. 2.2. Sampling devices. (A, D) Anchor dredge, drawing and potograph. (B-C) Higgins meiobenthic dredge, drawing and photograph. (E) Cylindrical collecting tool (15 cm diameter and 40 cm length) attached to a fisheries bottom trawl utilized for the campaign CARIOCA90 samplings conducted by the Spanish Institute of Oceanography.

2.5. Sample processing

After the collection of the sediment, the meiofauna was extracted following the "bubbling and blot" method (Higgins 1964; Higgins and Thiel 1988; Sørensen and Pardos 2008).

The aim of this method is basically to create as much turbulence and bubbles as possible in the sample in order to trap the "hard" meiofauna (meaning the meiofauna with a hard exocuticle). To do that the sediment sample was diluted and mixed with about twice its volume with sea water until getting the consistence as a thick soup. After that, the content of the bucket was vigorously poured back and forth into an empty bucket several times. During this process, the extremely hydrophobic exocuticle of Kinorhynchs and other animals is attracted and trapped by the created air bubbles driving them to the surface of the sample where they get stuck by the surface tension forming a foamy layer. Once in the surface, after letting the suspended particles to settle, the animals are already isolated from the sediment, and were collected with regular copying paper that was washed on top of a 63 µm mesh ("mermaid bra"). The whole process is repeated until nothing is left at the surface. This process allows getting a single tube of animals with a minimum part of sediment from a whole bucket of sediment. The bubbling technique is non-quantitative; however best estimates suggest that about 90-95% of the kinorhynchs can be removed by this method (Higgins and Thiel 1988). This method is only fully effective with fresh samples and seems not adequate with already fixed ones (personal observation).

2.6. Sorting of Kinorhynchs

Cyclorhagid kinorhynchs were separated from the remaining meiofauna in a Petri dish under a dissecting microscope utilizing a small tool named *Irwin loop* which is made up with thin metal wire mounted in a handle and twisted to form a minute loop in one of the tips. The usage of this tool is essential in the manipulation of specimens and assured a safe transfer into preservation vials and small observation dishes, or onto slides. The sorting process was done either before or after the fixation process. When after the fixation, the samples were pre-stained with Rose Bengal in order to facilitate the sorting.

2.7. Fixation and preservation

Different fixation methods were carried out in order to prepare the specimens for different studies. Samples for taxonomic studies were fixed and preserved in 7-10% Formalin. For morphologic studies the fixation was performed with 4% of Paraformaldehyde and preserved in 0.1 M Phosphate Buffered Saline (PBS) with sodium azide (NaN_3) to avoid the growth of microorganisms. For molecular purposes the samples were fixed and preserved in 99.9% of ethanol.

2.8. Mounting

Specimens studied with Light microscopy (LM) were dehydrated through a graded series of ethanol up to 96% and after transferred to a solution of 10% glycerin in 96% ethanol (Sørensen and Pardos 2008). This solution evaporated over night to glycerin. The mounting medium selected was Floromount-G® (Southern Biotech) due to his viscosity and absence of bleaching agents in its composition. The mounting was done in regular slides or alternatively in *cobb-slides*. The latter allow the observation of the specimen from both sides due to

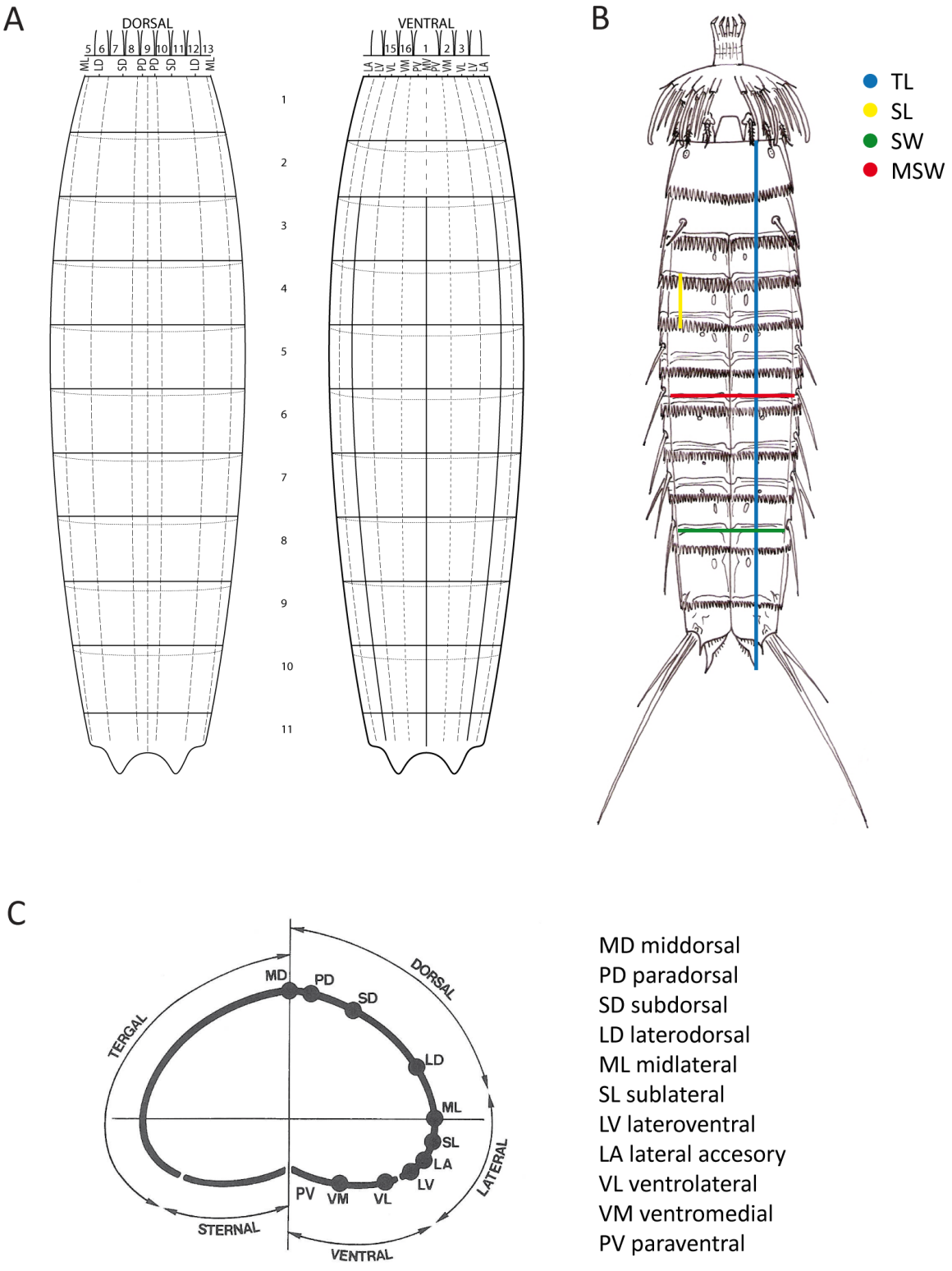


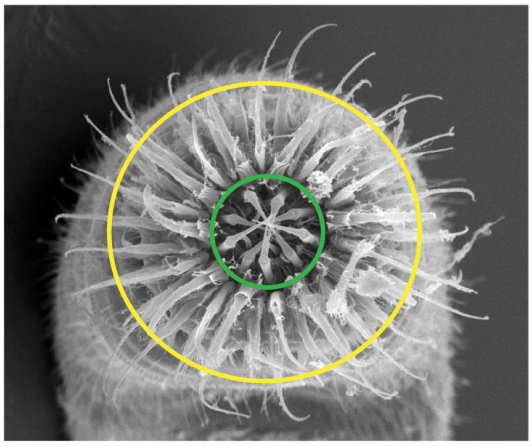
Fig. 2.3. Study of the trunk in cyclorhagid kinorhynchs. (A) Blind diagrams of dorsal (left) and ventral (right) views of the trunk of a cyclorhagid kinorhynch to plot in cuticular important cuticular structures, such as spines, sensory spots, glandular openings, etc. Solid lines indicate divisions between segments or segmental plates (except the solid outline). Dashed lines are positional guidelines following Pardos et al. (1998) and Thormar and Sørensen (2010). Diagram kindly provided by Jonas Thormar. (B) Trunk standard measurements for descriptions, modified from Sørensen and Pardos (2008). (C) Transverse section of a cyclorhagid kinorhynch showing positions of cuticular structures, modified from Sørensen and Pardos (2008). Abbreviations: MSW, maximum standard width; SL, segment length; SW, standard width; TL, total length.

the montage between two coverslips in an aluminum frame (Higgins 1971, 1988). A modern version of the cobb-slides is the Higgins-Shirayama slide (H-S slide) made with plastic instead of metal but not used due to its deterioration through time. After the mounting the slides were sealed with Depex®.

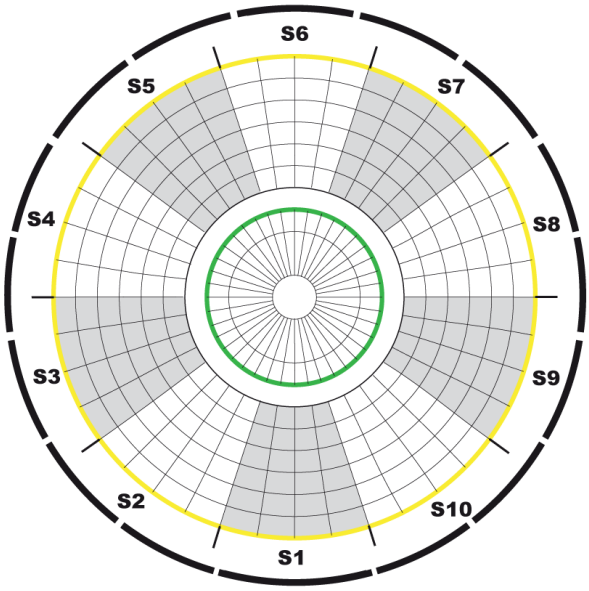
Specimens studied with SEM were dehydrated through a graded series of ethanol up to 100%, critical point dried and transferred to an aluminum stubs with adhesive carbon pads. After that, the specimens were sputter coated with either gold or platinum-palladium and examined with the corresponding scanning electron microscope. For more detailed descriptions on the equipment used see materials and methods of each publication.

Specimens studied with CLSM were washed in 0.1M PBS and permeabilize in PBT (PBS+ 0.5% Triton X-100) overnight. After this process the specimens were treated following different protocols depending on the target of the study. Each protocol is entirely detailed in the materials and methods of the corresponding publication. The mounting in most of the cases was carried out individually in regular slides, using clay feet to avoid the distortion of the specimen. The mounting media used were: Glicerol 80%, Vectashield® or Fluoromount® being Glicerol the more appropriate and less distorting for the specimens.

A



B



C

Ring/Section	1	2	3	4	5	6	7	8	9	10	Total
00 os											
01											
02 03 04 05 06 07 08 Tr											
Total scalids											

Fig. 2.4. Study of the introvert in kinorhynchs. (A) Apical view of *Echinoderes cantabricus*, showing in green the mouth cone and in yellow the introvert. (B) Blind diagram schematizing a polar view of the head to plot in the position and the appendages of the introvert and mouth cone. (C) Table to record the number of appendages per each section and row.

2.9. Study of the specimens

Taxonomical studies were carried out using an Olympus BX51 microscope with Nomarski differential interference contrast optics (DIC) equipped with an Olympus Colorview digital camera DP70. The specimens mounted for LM were examined, identified photographed and measured. In order to identify each specimen blind diagrammed drawings were used to plot in positions of taxonomic characters such as spines, tubules, glands, sensory spots, etc. (Fig.2.3. A). Measurements on LM pictures were made with Olympus Cell ^A software. Positional terminology follows that proposed by Pardos et al. (1998), Sørensen and Pardos (2008) and Thormar and Sørensen (2010) (Fig.2.3. B-C).

Light microscopy examinations were complemented with SEM studies providing details on the external anatomy of the trunk, neck and arrangement of the introvert and mouth cone appendages. The mapping of head appendages was made for each species utilizing blind schematized polar views of the head (Fig.2.4). Details on the SEM equipment used can be found in the corresponding publications.

Morphological studies were carried out using CLSM; this technique uses a laser instead of transmitted light allowing optical sectioning through the specimen. Images are acquired point-by-point and reconstructed with a computer, enabling three-dimensional reconstructions. All labeled specimens were analyzed and imaged using different confocal microscopes (see details in each publication). Optical sections and z-stack projection micrographs were compiled with Fiji, version 1.45 m (Wayne Rasband, National Institutes of Health). Image stacks were surface-rendered with the image editing software Imaris v. 7.5.0 (Bitplane AG, Zürich, Switzerland) to create the 3D models.

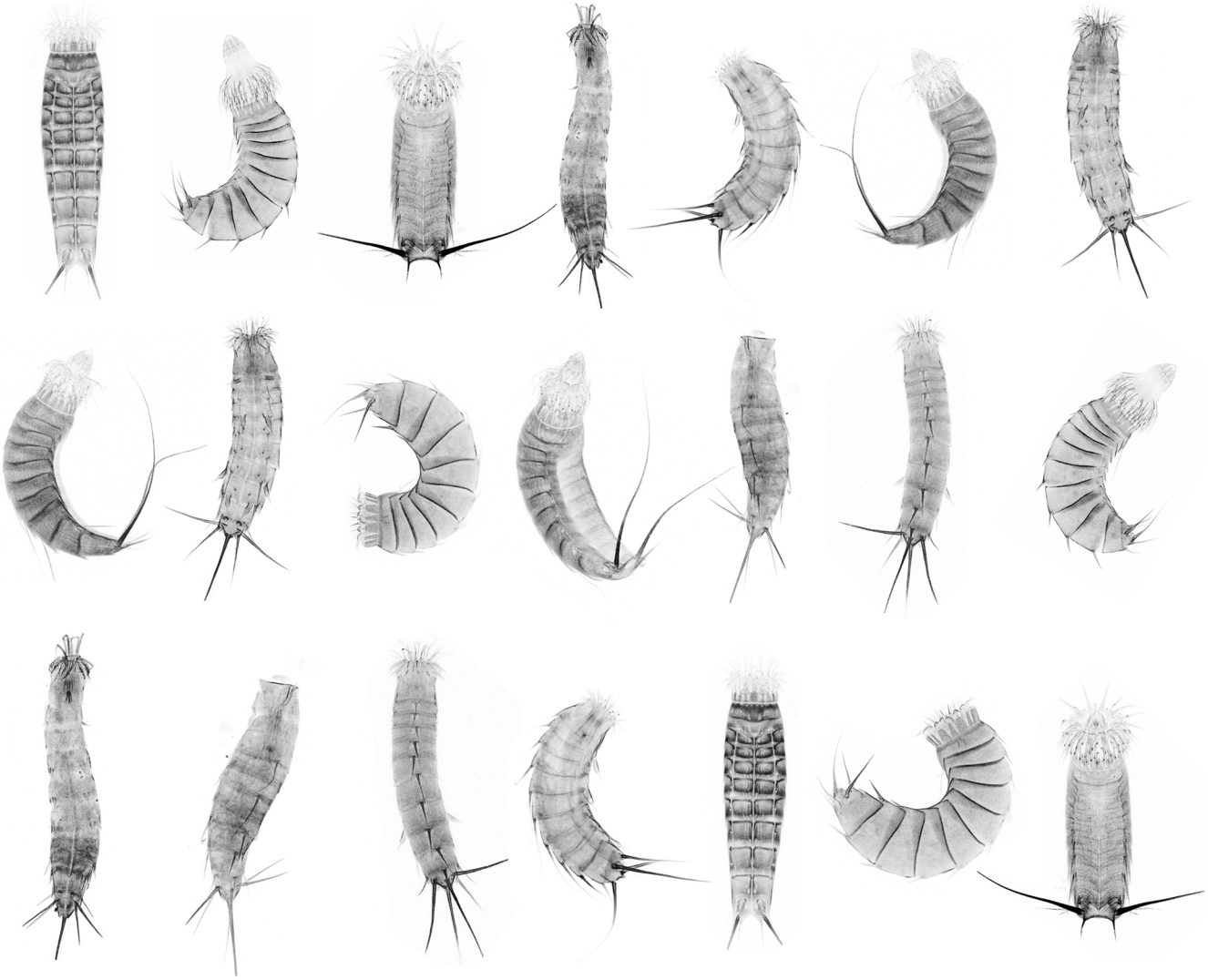
Schematics and figure plates were prepared with Adobe Illustrator CS4 (Adobe Systems Incorporated, San Jose, CA).

What is a scientist after all? It is a curious man looking through a keyhole, the keyhole of nature, trying to know what's going on.

Jacques Yves Cousteau

3

Results
Biogeography



Chapter I

Phylum Kinorhyncha from the Iberian Peninsula: new data from the first intensive sampling campaigns

El filo Kinorhyncha en la Península Ibérica: nuevos datos de la primera campaña intensiva de muestreos en una línea de costa

Nuria Sánchez, MARÍA HERRANZ, Jesús Benito y Fernando Pardos
Zootaxa 3402: 24–44 (2012)

En este trabajo se presentan los datos de los primeros muestreos intensivos del filo Kinorrincos realizados en la Península Ibérica a lo largo de un período de 21 años, desde 1990 a 2011 en 81 localidades y generalmente a profundidades menores de 100 m. El examen con microscopía óptica de los ejemplares obtenidos resultó en la identificación de 11 géneros y 29 especies, de las que solo 11 se habían citado anteriormente en aguas peninsulares. Se registran diez nuevas especies para la Península y ocho nuevas especies aún sin describir. El género más rico es *Echinoderes* con diez especies, dos de ellas nuevas, seguido por *Pycnophyes* con nueve especies, tres de ellas nuevas. Se han encontrado dos especies de *Antygomonas* (una nueva para la ciencia), y una especie de cada uno de los géneros *Campyloderes, Centroderes, Condyloderes* (una especie nueva para la ciencia), *Dracoderes, Meristoderes, Semnoderes, Kinorhynchus* (una especie nueva para la ciencia) y *Paracentrophyes*. La especie más ubicua en las muestras y que aparece abundantemente en casi todas las localidades fue *Pycnophyes dentatus*, una nueva cita para la Península Ibérica. *Echinoderes cantabricus, E. hispanicus* y *E. dujardinii* también tienen una amplia distribución a lo largo de las costas mediterráneas y atlánticas. Aunando los datos obtenidos junto con datos previos de la zona se discute sobre la diversidad, la biogeografía y la ecología (profundidad, naturaleza del sedimento y abundancia) del filo en aguas españolas.

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Kinorhyncha from the Iberian Peninsula: new data from the first intensive sampling campaigns

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Abstract

Data are presented from the first intensive sampling of Kinorhyncha around the Iberian Peninsula over a 21-year period from 1990 to 2011, from 81 sites mostly in less than 100 m water depth. Light-microscopic examination of approximately 2000 specimens yielded 11 genera and 29 species, only 11 of which were previously recorded from peninsular waters. The balance comprises ten new species records for the peninsula and eight new species that are yet to be described. The most speciose genus is *Echinoderes*, with ten species, two of them new, followed by *Pycnophyes* (nine species, three new). There are two species of *Antygomonas* (one new), and one each for the genera *Campyloderes*, *Centroderes*, *Condyloderes* (one new), *Dracoderes*, *Meristoderes*, *Semnoderes*, *Kinorhynchus* (one new), and *Paracentrophyes*. The most ubiquitous species in the samples, appearing at nearly all localities was *Pycnophyes dentatus*, newly recorded for the Iberian Peninsula and found at nearly all sampled localities and in high numbers. *Echinoderes cantabricus*, *E. hispanicus* and *E. dujardinii* also have a wide distribution along both Atlantic and Mediterranean coasts. Known information on diversity, biogeography and ecology (depth, sediment and abundance) is discussed.

Key words: Kinorhyncha, meiofauna, biogeography, distribution, sampling, Iberian Peninsula

Introduction

Kinorhyncha is a phylum of ecdysozoan meiobenthic organisms less than 1 mm in length and found exclusively in marine or estuarine sediments, from coarse sand or shell gravel to very fine mud (Higgins 1988). Their general anatomy and taxonomic characters are well described by Higgins (1988), Kristensen & Higgins (1991) and Sørensen & Pardos (2008). The first known kinorhynch was described by Dujardin (1851) from Saint Malo, Atlantic coast of France. Subsequent comprehensive reviews of Kinorhyncha, including the fauna of the Mediterranean and Atlantic European coasts, were made by Zelinka (1928), Adrianov & Malakhov (1999) and Sørensen & Pardos (2008). The sampling effort reported in the present study has been paralleled only along the east coast of North America where 17 species from five genera have been found (Chitwood 1951; Higgins 1964a,b, 1965, 1977, 1982, 1990; Sørensen *et al.* 2005, 2007; Sørensen & Pardos 2008), but in recent years the waters around the Korean Peninsula have been fairly intensively surveyed, yielding ten species from six genera to date (Song & Chang 2001; Lundbye *et al.* 2010; Sørensen *et al.* 2010a-c; Sørensen *et al.* 2012a; Sørensen 2012b).

Studies of kinorhynchs from the Iberian Peninsula are scarce. As a group, they have been noted mainly in ecological studies, unaccompanied by descriptions or identifications to genus or species (e.g. Villora 1993). Subsequently, five species of cyclorhagid kinorhynchs belonging to the genus *Echinoderes* were described from the north coast of Spain, including the new taxa *E. hispanicus* Pardos *et al.*, 1998 and *E. cantabricus* Pardos *et al.*, 1998 from Santoña Bay (Cantabria). Ten years later, three additional species were described from several sampling localities ranging from Ribadeo (Asturias) to Bidasoa (Guipúzcoa), northern Spain: *Echinoderes isabelae* GªOrdóñez *et al.*, 2008, *E. parrai* GªOrdóñez *et al.*, 2008 and *E. neospinosus* GªOrdóñez *et al.*, 2008.

Recently, Sørensen *et al.* (2010a) reported the homalorhagid *Paracentrophyes quadridentatus* (Zelinka, 1928) from the Cantabrian coast. Moreover, two new homalorhagids, *Pycnophyes dolichurus* Sánchez *et al.*, 2011 and *P.*

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aulacodes Sánchez *et al.*, 2011 were described from Galician waters, northwestern Spain, the latter being reported also from several Mediterranean localities. In addition, a new species of *Dracoderes*, *D. gallaicus* Sørensen *et al.*, 2012 (2012a), was described from Ría de Arosa, Ría de Vigo and Algeciras and *Echinoderes dujardinii* Claperède, 1863 was found at Almuñécar (Granada, south Spain) by Sánchez-Tocino *et al.* (2011). Finally, a new cyclorhagid genus and species, *Meristoderes macracanthus* Herranz *et al.*, 2012 was described from a Mediterranean locality.

Intensive sampling along the Iberian coasts reported herein has revealed a high kinorhynch biodiversity. This report brings together all published records of Iberian kinorhynchs, along with new records and distributional data, providing for the first time a catalogue of the entire known Kinorhyncha for the region.

Material and methods

Material for the present study was acquired through numerous collecting campaigns in the period 1990 through 2011. Samples from Asturias, San Vicente de la Barquera, Comillas and Bilbao were taken by the RV *Cornide de Saavedra* during the fisheries campaign CARIOCA90, conducted by the Spanish Institute of Oceanography, using a cylindrical collecting tool (15 cm diameter and 40 cm length) attached to a fisheries bottom trawl (GªOrdóñez *et al.* 2008). All other samples were collected with a Higgins Meiobenthic Dredge (Higgins 1964c, 1988; Pardos *et al.* 1998). Kinorhynchs were extracted from the sediment using the 'bubble and blot' procedure (see Higgins 1988; Sørensen & Pardos 2008), fixed in 7% formalin and stained with Rose Bengal to facilitate sorting.

The sampled localities along the Iberian coasts can be grouped into 13 areas, eight along the Atlantic coast and five along the Mediterranean coast: Bilbao, Cantabria (Castro-Urdiales, Santoña, Comillas and San Vicente de la Barquera), East Asturias (Deva mouth and Llanes), West Asturias (Cabo Peñas, Cudillero and Navia), North Galicia (Ría de Ares, Ría de Ferrol and Ría de La Coruña), South Galicia (Ría de Arosa, Ría de Pontevedra and Ría de Vigo), Gulf of Cádiz (Punta Umbría and Cádiz), Algeciras Bay, Granada (Almuñécar), Almería (Garrucha), Murcia (Isla Grosa), Valencia (Denia) and Gerona (Blanes) (Fig.1 and Tables 1, 2). For the purpose of this paper we follow the general biogeographic approach of including Algeciras Bay in the Atlantic sector.

About 2000 kinorhynch specimens were sorted under a dissecting microscope using an Irwin loop and mounted for light microscopy on regular slides or on Cobb-slides with either Hoyer's medium or Fluoromount-G®, following standard procedures (Sørensen & Pardos 2008). The specimens were observed and photographed using an Olympus BX51 microscope equipped with differential interference optics and an Olympus DP70 camera. Selected specimens (around 300) were dehydrated through a graded series of ethanol, transferred to acetone, critical-point dried, mounted on aluminum stubs and sputter-coated with gold for observation and photography with a JEOL JSM 6400 and 6335 field-emission SEM.

Physicochemical data for sediment samples are not homogeneous and sometimes unavailable because samples were taken over a long period (1990–2011; 21 years) and under very different circumstances. For example, in terms of sediment granulometry, standard procedures were not always applied, and hence we present here approximate, subjective data on sediment nature and grain size, grouped into eight categories often used in meiofaunal studies (Table 3).

Results

A total of 29 species in 11 genera and seven families were recorded along the Iberian coastline. Following the subdivision into 13 areas (boldface) and localities the species can be grouped as follows (Fig. 1):

Bilbao. Three species in three genera and three families: *Centroderes spinosus* (Reinhard, 1881) Zelinka, 1928 (Figs 2C, E), *Semnoderes armiger* Zelinka, 1928 (Figs 2B, 4G, K) and *Paracentrophyes quadridentatus*.

Cantabria. Six species in five genera and four families.

- Off Castro-Urdiales: *Centroderes spinosus* (Figs 2C, E), *Paracentrophyes quadridentatus* and *Semnoderes armiger* (Figs 2B, 4G, K).
- Santoña: *Echinoderes cantabricus* (Figs 4C, H) and *E. hispanicus*.
- Off Comillas: *C. spinosus* and *S. armiger*.

- Off San Vicente de la Barquera: *C. spinosus*, *Condyloderes* sp. 1 and *S. armiger.*

East Asturias (Deva mouth-Llanes). Eight species in six genera and five families.

- Off Deva mouth: eight species: *Centroderes spinosus* (Figs 2C, E), *Condyloderes* sp. 1, *Echinoderes isabelae*, *E. neospinosus, E. parrai*, *Semnoderes armiger* (Figs 2B, 4G, K), *Paracentrophyes quadridentatus* and *Pycnophyes aulacodes* (Figs 4B, F).
- Off Llanes: the same species as in Deva mouth except for the homalorhagids *P. quadridentatus* and *P. aulacodes.*

West Asturias (Cabo Peñas-Cudillero-Navia). Nine species in five genera and five families.

- Off Cabo Peñas (Gijón): two cyclorhagid and two homalorhagid species: *Centroderes spinosus* (Figs 2C, E), *Echinoderes isabelae*, *Pycnophyes aulacodes* (Figs 4B, F) and *Pycnophyes dentatus* (Reinhard, 1881) (Zelinka 1928) (Figs 4A, J).
- Off Cudillero: six species: *C. spinosus*, *E. isabelae, Echinoderes* sp. 1, *Semnoderes armiger* (Figs 2B, 4G, K), *Paracentrophyes quadridentatus* and *Pycnophyes dentatus*.
- Off Navia: eight species: *C. spinosus*, *E. isabelae*, *E. neospinosus*, *E. parrai*, *S. armiger*, *P. quadridentatus*, *P. aulacodes* and *P. dentatus.*

North Galicia. Six species in two genera and two families.

- Ría de Ferrol: two species of *Echinoderes*: *E. cantabricus* (Figs 4C, H) and *E. hispanicus*; and three species of *Pycnophyes*: *P. aulacodes* (Figs 4B, F), *P. dentatus* (Figs 4A, J) and *Pycnophyes* sp. 1.
- Ría de Ares: four species of *Pycnophyes*: *P. aulacodes* (Figs 4B, F), *P. dentatus*, *P. dolichurus* and *Pycnophyes* sp. 1.
- Ría de La Coruña: Same species of *Pycnophyes* as in Ría de Ferrol.

South Galicia. Ten species in four genera and four families.

- Ría de Arosa: nine species: *Antygomonas* sp. 1, *Dracoderes gallaicus*, *Echinoderes cantabricus* (Figs 4C, H), *E. dujardinii* (Fig. 2D), *E. hispanicus*, *Echinoderes kristenseni* Higgins, 1985, *Echinoderes* sp. 1, *Pycnophyes aulacodes* (Figs. 4B, F) and *P. dentatus* (Figs 4A, J).
- Ría de Pontevedra, Combarro: three species belonging to two genera: *E. cantabricus* (Figs 4C, H), *P. aulacodes* and *P. dentatus.*
- Ría de Vigo: six different species: *Antygomonas* sp. 1, *D. gallaicus*, *E. cantabricus, P. aulacodes*, *P. dentatus* and *Pycnophyes* sp. 1.

Gulf of Cádiz. Five species belonging to two genera and two families.

- Punta Umbría: three cyclorhagid species and one homalorhagid species: *Echinoderes cantabricus* (Figs 4C, H), *E. hispanicus*, *Echinoderes worthingi* Southern, 1914 and *Pycnophyes dentatus* (Figs 4A, J).
- Cádiz: same cyclorhagids as in Punta Umbría and only one homalorhagid, *Pycnophyes* sp. 2.

Algeciras Bay. Eleven species in six genera and four families*: Campyloderes* cf. *vanhoeffeni* Zelinka, 1913 (Figs 2A, 4D), *Centroderes spinosus* (Figs 2C, E), *Dracoderes gallaicus*, *Echinoderes cantabricus* (Figs 4C, H), *E. dujardinii* (Fig. 2D), *E. hispanicus*, *Echinoderes* sp. 2, *Kinorhynchus* sp. 1, *Pycnophyes dentatus* (Figs 4A, J), *Pycnophyes zelinkaei* Southern, 1914 (Figs 3A, D, E) and *Pycnophyes* sp. 1.

Granada (Almuñécar). Nine species in two genera and two families: *Echinoderes cantabricus* (Figs 4C, F), *E. dujardinii* (Fig. 2D), *E. hispanicus*, *Echinoderes* sp. 1, *Echinoderes* sp. 2, *Pycnophyes aulacodes* (Figs 4B, F), *Pycnophyes carinatus* Zelinka, 1928 (Figs 3 B-C, 4E, I), *P. dentatus* (Figs 4A, J) and *Pycnophyes* sp. 1.

Almería (Garrucha). Three species from two genera and two families: *Echinoderes hispanicus*, *Pycnophyes aulacodes* (Figs 4B, F) and *Pycnophyes communis* Zelinka, 1908.

Murcia (Isla Grosa). Three species in two genera and two families: *Campyloderes* cf. *vanhoeffeni* (Figs 2A, 4D), *Echinoderes dujardinii* (Fig. 2D) and *E. hispanicus*.

Alicante (Denia). Seven species from four genera and four families: *Campyloderes* cf. *vanhoeffeni* (Figs 2A, 4D), *Echinoderes dujardinii* (Fig. 2D), *Echinoderes* sp. 1, *Semnoderes armiger* (Figs 2B, 4G, K), *Pycnophyes aulacodes* (Figs 4B, F), *P. communis* and *P. dentatus* (Figs 4A, J).

Gerona (Blanes). Thirteen species in six genera and five families: *Antygomonas incomitata* Nebelsick, 1990, *Centroderes spinosus* (Figs 2C, E), *Echinoderes hispanicus*, *E. isabelae*, *Echinoderes* sp. 1, *Meristoderes macracanthus*, *Semnoderes armiger* (Figs 2B, 4G, K), *Pycnophyes aulacodes*, *P. carinatus* (Figs 3 B-C, 4E, I), *P. communis*, *P. dentatus* (Figs 4A, J), *P. zelinkaei* (Figs 3A, D, E) and *Pycnophyes* sp. 3.

Data are summarized by taxon in Table 2, by distribution in Figure 5 and by diversity levels in Figure 6.

TABLE 1. Sampling stations referred to in this study and the number of specimens extracted from sediment samples. Not all specimens were been mounted on slides for study. Some localities yielded a large quantity of specimens that were not counted beyond a certain point; these are marked with a + symbol. Abbreviations: Cyclor., Cyclorhagida; Homalor., Homalorhagida; n, number of specimens; N/A, no available data.

Area	Locality	Date	Sample	Coordinates	Sediment	Depth	*n* Homalor.	*n* Cyclor.
Bilbao	Off Bilbao	90.10.24	3.35	43º31.90' N; 2º01.76' W	N/A	174 m	0	19
Bilbao	Off Bilbao	90.10.24	3.39	43º31.30' N; 2º26.30' W	N/A	226 m	0	1
Bilbao	Off Bilbao	90.10.24	3.38	43º32.70' N; 2º32.80' W	N/A	180 m	0	6
Bilbao	Off Bilbao	90.10.24	3.47	43º35.20' N; 3º04.70' W	N/A	188 m	0	52
Bilbao	Off Bilbao	90.10.24	3.46	43º35.60' N; 3º05.20' W	Muddy sand	238 m	10	20
Cantabria	Off Castro-Urdiales	90.10.24	3.44	43º35.70' N; 3º12.20' W	Muddy sand	272 m	4	112
Cantabria	Off Castro-Urdiales	90.10.24	3.45	43º34.63' N; 3º17.80' W	Muddy sand	192 m	4	9
Cantabria	Santoña	90.08.30/ 91.03.04	1	43°26' N; 3°28' W	Sandy mud	3-15 m	N/A	N/A
Cantabria	Off Comillas	90.10.24	2.63	43º28.50' N; 4º14.30' W	N/A	111 m	0	5
Cantabria	Off Comillas	90.10.24	2.65	43º28.80' N; 4º23.10' W	N/A	134 m	0	8
Cantabria	Off S. Vicente de la Barquera	90.10.24	2.55	43º41.60' N; 4º32.60' W	N/A	308 m	0	24
Cantabria	Off S. Vicente de la Barquera	90.10.24	2.61	43º30.60' N; 4º32.70' W	N/A	244 m	0	139
Cantabria	Off S. Vicente de la Barquera	90.10.24	2.58	43º38.60' N; 4º36.70' W	N/A	188 m	0	3
East Asturias	Off Deva mouth	90.10.24	2.56	43º39.30' N; 4º49.30' W	Muddy sand	191 m	22	40
East Asturias	Off Deva mouth	90.10.24	2.57	43º39.48' N; 4º39.80' W	Muddy sand	179 m	30	46
East Asturias	Off Deva mouth	90.10.24	2.60	43º35.00' N; 4º40.00' W	Muddy sand	168 m	132	45
East Asturias	Off Deva mouth	90.10.24	2.59	43º29.10' N; 4º43.00' W	Muddy sand	107 m	7	33
East Asturias	Off Llanes	90.10.24	2.31	43º38.46' N; 5º00.30' W	N/A	444 m	0	7
East Asturias	Off Llanes	90.10.24	2.29	43º46.99' N; 5º03.32' W	N/A	435 m	0	25
East Asturias	Off Llanes	90.10.24	2.30	43º45.20 N; 5º03.60 W	N/A	176 m	0	89
West Asturias	Off Cabo Peña	90.10.24	2.22	43º44.20' N; 5º40.00' W	N/A	95 m	42	5
West Asturias	Off Cudillero	90.10.24	1.8	43º47.70' N; 6º02.40' W	Muddy sand	168 m	2	130
West Asturias	Off Cudillero	90.10.24	1.9	43º35.40' N; 6º06.50' W	N/A	49 m	47	4
West Asturias	Off Navia	90.10.24	1.13	43º55.30' N; 6º36.70' W	N/A	79 m	0	49
West Asturias	Off Navia	90.10.24	1.12	43º53.40' N; 6º36.80' W	N/A	140 m	0	17
West Asturias	Off Navia	90.10.24	1.7	43º35.60' N; 6º40.50' W	N/A	76 m	178	902

Continued next page

TABLE 1 (continued)

Area	Locality	Date	Sample	Coordinates	Sediment	Depth	*n* Homalor.	*n* Cyclor.
West Asturias	Off Navia	90.10.24	1.10	43º58.30' N; 6º43.50' W	N/A	369 m	0	3
West Asturias	Off Navia	90.10.24	1.6	43º35.60' N; 6º43.80' W	N/A	71 m	0	165
West Asturias	Off Navia	90.10.24	1.11	43º58.20' N; 6º45.00' W	N/A	304 m	0	70
West Asturias	Off Navia	90.10.24	1.17	43º44.30' N; 7º00.20' W	Muddy sand	127 m	22	93
North Galicia	Ría de Ferrol, Rabo de Porca	07.06.26	1A	43°27.523' N; 8°17.707' W	Shell gravel	N/A	0	2
North Galicia	Ría de Ferrol, Castelo de Palma	07.06.26	3A	43°27.719' N; 8°16.700' W	*Amphioxus* sand (Coarse sand)	N/A	3	2
North Galicia	Ría de Ferrol	07.06.26	5A	43°28.178' N; 8°14.716' W	Mud	N/A	40	20
North Galicia	Ría de Ferrol, San Cristobo	07.06.27	5B	43°27.887' N; 8°18.118' W	Muddy sand	N/A	27	30
North Galicia	Ría de Ares	07.06.27	1A	43°25.064' N; 8°16.558' W	Muddy sand	N/A	46	0
North Galicia	Ría de Ares	07.06.27	3A	43°24.844' N; 8°17.832' W	Coarse sand	N/A	30	0
North Galicia	Ría de Ares	08.04.04	3B	43°25.400' N; 8°20.769' W	Fine sand	45.4 m	100+	0
North Galicia	Ría de Ares	08.04.04	5B	43°23.232' N; 8°15.391' W	Fine sand	13 m	30	0
North Galicia	Ría de La Coruña	08.04.03	1A	43°21.046' N; 8°22.245' W	Fine sand	7.9 m	39	0
North Galicia	Ría de La Coruña	08.04.03	3A	43°22.208' N; 8°21.177' W	Fine sand	19 m	19	0
North Galicia	Ría de La Coruña	08.04.03	4A	43°22,718' N; 8°21.858' W	Fine sand	27 m	9	0
North Galicia	Ría de La Coruña	08.04.03	6A	43°21.697' N; 8°22.713' W	Muddy sand	19.1 m	2	0
North Galicia	Ría de La Coruña	08.04.04	2B	43°24.981' N; 8°22.853' W	Fine sand	53 m	4	0
South Galicia	Ría de Arosa	09.04.24	2	42°33.242' N; 8°56.357' W	Mud	28 m	13	17
South Galicia	Ría de Arosa	09.04.24	3	42°32.663' N; 8°54.00 W	Fine sand	3 m	0	13
South Galicia	Ría de Arosa	09.04.24	4	42°34.134' N; 8°53.580 W	Maërl	8 m	0	15
South Galicia	Ría de Arosa	09.04.24	5	42°32.795' N; 8°51.032' W	Mud	3 m	3	2
South Galicia	Ría de Arosa	09.04.24	6	42°32.768' N; 8°51.103' W	Shell gravel	1.6 m	0	2
South Galicia	Ría de Pontevedra, Combarro	08.11.14	1	42º26'00.66'' N; 8º41'50.78'' W	Mud	Intertidal	130	108
South Galicia	Ría de Vigo	09.04.25	1	42°7.833' N; 8°50.248' W	*Amphioxus* sand (Coarse sand)	10 m	0	56
South Galicia	Ría de Vigo	09.04.25	2	42°14.640' N; 8°51.487' W	Fine sand and shell gravel	22.5 m	5	22
South Galicia	Ría de Vigo	09.04.25	3	42°15.057' N; 8°51.544' W	Fine sand	7.5 m	0	75
South Galicia	Ría de Vigo	09.04.25	6	42°14.089' N; 8°45.564' W	Mud	21 m	23	68
Gulf of Cádiz	Punta Umbría	93.09.24	4	37º8'59.87'' N; 6º57'34.57'' W	Mud	N/A	14	N/A

Continued next page

TABLE 1 (continued)

Area	Locality	Date	Sample	Coordinates	Sediment	Depth	*n* Homalor.	*n* Cyclor.
Gulf of Cádiz	La Cortadura	93.09.23	2	36º31'34.95'' N; 6º13'31.75'' W	Mud	N/A	19	N/A
Algeciras Bay	Algeciras	93.09.26	1.1	36º10'10.02'' N; 5º23'10.49'' W	Muddy sand	80 m	0	1
Algeciras Bay	Algeciras	11.02.07	2A	36º05.354' N; 5º26.170' W	Muddy sand	8-10 m	11	0
Algeciras Bay	Algeciras	11.02.07	4A	36º05.805' N; 5º26.284' W	Fine sand and shell gravel	30 m	6	55+
Algeciras Bay	Algeciras	11.02.07	5A	36º07'22.97'' N; 5º25'11.45'' W	Fine sand and Shell gravel	24 m	0	36
Algeciras Bay	Algeciras	11.02.08	1B	36º09.272' N; 5º26.296' W	Mud	12 m	65	27
Algeciras Bay	Algeciras	11.02.08	2B	36º10.348' N; 5º26.464' W	Fine mud	15-17 m	12	35
Algeciras Bay	Guadarranque mouth	11.02.08	3B	36º10.583' N; 5º24.620' W	Fine mud	25 m	12	9
Algeciras Bay	Algeciras	11.02.08	4B	36º10.741' N; 5º23.243' W	Sandy mud	8 m	50+	10
Algeciras Bay	La línea	11.02.08	5B	36º09.630' N; 5º22.256' W	Mud	12 m	5	98
Granada	Almuñécar	10.07.24	1	36º44'03.09'' N; 3º45'39.61" W	Mud	30 m	10	0
Granada	Almuñécar	10.07.24	2	36º43'32.54'' N; 3º44'22.97'' W	Muddy sand	15 m	30	0
Granada	Almuñécar	10.07.24	3	36º43'42.70'' N; 3º43'17.37'' W	Coarse sand	18 m	53	28
Granada	Almuñécar	10.07.24	4	36º43'45.47'' N; 3º42'47.76'' W	Mud	20 m	55	0
Almería	Garrucha	97.03.24	1	37º9'13.97'' N; 1º47'57.12'' W	Coarse sand	15 m	21	0
Almería	Garrucha	97.03.24	2	37º11'40.68' 'N; 1º48'1.81'' W	N/A	8 m	10	30
Almería	Garrucha	97.03.25	3	37º10'55.53'' N; 1º49'30.3'' W	Mud	4 m	6	0
Murcia	Isla Grosa	10.03.27	1	37º43'32.98'' N; 0º 42'26.04'' W	Coarse sand	10 m	0	21
Alicante	Denia	97.03.27	1	38º51'2.77'' N; 0º8'10'' W	Mud	15 m	2	4
Alicante	Denia	97.03.27	2	38º50'14'' N; 0º9'25'' W	Fine sand	12 m	14	10
Alicante	Denia	97.03.27	3	38º49'6'' N; 0º11'30'' W	N/A	12 m	10	0
Gerona	Blanes	99.03.24	1	41º40.210' N; 02º47.740' W	N/A	11.6 m	0	22
Gerona	Blanes	99.03.24	2	41º39.326' N; 2º47.000' W	N/A	11 m	0	50+
Gerona	Blanes	99.03.24	3	41º38.596' N; 2º46.255' W	Fine sand	28.4 m	20	52
Gerona	Blanes	99.03.24	4	41º38.511' N; 2º46.322' W	Muddy sand	37.5 m	20	100+
Gerona	Blanes	99.03.24	5	41º39.386' N; 2º48.341' W	*Dentalium* sand (Coarse sand)	55.4 m	0	29
Gerona	Blanes	99.03.24	6	41º39.420' N; 2º47.970' W	Fine sand	30 m	0	9

TABLE 2. Diversity and distribution of Kinorhyncha along the Iberian Peninsula based on published data and the present study. Species are marked as new records for the Mediterranean Sea, Atlantic Ocean and the Iberian Peninsula. Abbreviations: Atl, new citation for the Atlantic Ocean; Med, new citation for the Mediterranean Sea; Ib. P., new citation for the Iberian Peninsula; * this paper.

Genus	Species	Locality	Sample	Reference	Med	Atl	Ib. P.
Antygomonas	*A. incomitata*	Blanes	2,6	*			X
	A. sp.1	Ría de Arosa	3, 4	*		X	X
		Ría de Vigo	1, 2, 3	*			
Campyloderes	*C.* cf. *vanhoeffeni*	Algeciras Bay	1.1	*	X		X
		Isla Grosa	1	*			
		Denia	2	*			
Centroderes	*C. spinosus*	Bilbao	3.46	*			X
		Castro-Urdiales	3.44, 3.45	*			
		Comillas	2.63, 2.65	*			
		San Vicente	2.55, 2.58, 2.61	*			
		Deva mouth	2.56,2 .59, 2.60	*			
		Llanes	2.29, 2.30, 2.31	*			
		Cabo Peñas	2.22	*			
		Cudillero	1.8, 1.9	*			
		Navia	1.6, 1.7, 1.10, 1.11, 1.13, 1.17	*			
		Algeciras Bay	1.1	*			
		Blanes	5	*			
Condyloderes	*C.* sp.1	San Vicente	2.55, 2.61	*		X	X
		Deva mouth	2.56, 2.57, 2.60	*			
		Llanes	2.30	*			
Dracoderes	*D. gallaicus*	Ría de Arosa		Sørensen *et al.* 2012a			
		Ría de Vigo		Sørensen *et al.* 2012a			
		Algeciras Bay		Sørensen *et al.* 2012a			
Echinoderes	*E. cantabricus*	Santoña		Pardos *et al.* 1998	X		
		Ría de Ferrol	5A	*			
		Ría de Arosa	2	*			
		Ría de Pontevedra	1	*			
		Ría de Vigo	2, 6	*			
		Punta Umbría	4	*			
		Gulf of Cádiz	2	*			
		Algeciras Bay	4A, 5A, 1B, 2B, 3B	*			
		Almuñécar	3	*			
		Garrucha	2	*			
		Isla Grosa	1	*			

Continued next page…

TABLE 2. (continued)

Genus	Species	Locality	Sample	Reference	Med	Atl	Ib. P.
Echinoderes	*E. dujardinii*	Ría de Arosa	6	*			
		Algeciras bay	4B, 5B	*			
		Almuñécar	3	Sánchez-Tocino *et al.* 2011			
		Denia	1	*			
		Isla Grosa	1	*			
	E. hispanicus	Santoña		Pardos *et al.* 1998	X		
		Ría de Ferrol	5A	*			
		Ría de Arosa	5	*			
		Punta Umbría	4	*			
		Gulf of Cádiz	2	*			
		Algeciras Bay	4A, 5A, 1B, 3B, 4B, 5B	*			
		Almuñécar	3	*			
		Garrucha	2	*			
		Isla Grosa	1	*			
		Blanes	3, 4, 6	*			
	E. isabelae	Bilbao		GªOrdóñez *et al.* 2008	X		
		Comillas		GªOrdóñez *et al.* 2008			
		Deva mouth		GªOrdóñez *et al.* 2008			
		Llanes		GªOrdóñez *et al.* 2008			
		Cabo Peñas		GªOrdóñez *et al.* 2008			
		Cudillero		GªOrdóñez *et al.* 2008			
		Navia		GªOrdóñez *et al.* 2008			
		Blanes	3,4	*			
	E. kristenseni	Ría de Arosa	4	*			X
	E. neospinosus	Bilbao		GªOrdóñez *et al.* 2008			
		San Vicente		GªOrdóñez *et al.* 2008			
		Deva mouth		GªOrdóñez *et al.* 2008			
		Llanes		GªOrdóñez *et al.* 2008			
		Navia		GªOrdóñez *et al.* 2008			
	E. parrai	Deva mouth		GªOrdóñez *et al.* 2008			
		Llanes		GªOrdóñez *et al.* 2008			
		Navia		GªOrdóñez *et al.* 2008			
	E. worthingi	Ría de Ferrol	1A, 3A	*			X
		Punta Umbría	4	*			
		Gulf of Cádiz	2	*			
	E. sp.1	Cudillero	1.8	*	X	X	X
		Ría de Arosa	2	*			
		Almuñécar	3	*			

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TABLE 2. (continued)

Genus	Species	Locality	Sample	Reference	Med	Atl	Ib. P.
Echinoderes	*E.* sp.1	Denia	2	*			
		Blanes	3, 4	*			
	E. sp.2	Algeciras Bay	4A, 5A	*	X	X	X
		Almuñécar	3	*			
Meristoderes	*M. macracanthus*	Blanes		Herranz *et al.* 2012			
Semnoderes	*S. armiger*	Bilbao	3.35,3.38,3.39,3.46,3.47	*			X
		Castro-Urdiales	3.45	*			
		Comillas	2.65	*			
		San Vicente	2.55,2.61	*			
		Deva mouth	2.56,2.57,2.59,2.60	*			
		Llanes	2.30,2.31	*			
		Cudillero	1.8,1.9	*			
		Navia	1.6,1.7,1.11,1.12, 1.13,1.17	*			
		Denia	1	*			
		Blanes	5	*			
Paracentrophyes	*P. quadridentatus*	Bilbao	3.46	*			
		Castro-Urdiales	3.44, 3.45	*			
		Deva mouth		Sørensen *et al.* 2010a			
		Cudillero	1.8	*			
		Navia	1.17	*			
Kinorhynchus	*K.* sp.	Algeciras Bay	2B	*		X	X
Pycnophyes	*P. aulacodes*	Deva mouth	2.60	*			
		Cabo Peñas	2.22	*			
		Navia	1.7, 1.17	*			
		Ría de Ares		Sánchez *et al.* 2011			
		Ría de Ferrol		Sánchez *et al.* 2011			
		Ría de La Coruña		Sánchez *et al.* 2011			
		Ría de Arosa	2	*			
		Ría de Pontevedra	1	*			
		Ría de Vigo	6	*			
		Almuñécar	2,3,4	*			
		Garrucha		Sánchez *et al.* 2011			
		Denia		Sánchez *et al.* 2011			
		Blanes		Sánchez *et al.* 2011			
	P. carinatus	Almuñécar	3, 4	*			X
		Blanes	4	*			

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TABLE 2. (continued)

Genus	Species	Locality	Sample	Reference	Med	Atl	Ib. P.
Pycnophyes	*P. communis*	Garrucha	1, 3	*			X
		Denia	2	*			
		Blanes	4	*			
	P. dentatus	Cabo Peñas	2.22	*			X
		Cudillero	1.9	*			
		Navia	1.7	*			
		Ría de Ferrol	3A, 5A, 5B	*			
		Ría de Ares	1A, 3A, 3B, 5B	*			
		Ría de La Coruña	1A, 3A, 4A, 2B	*			
		Ría de Arosa	5	*			
		Ría de Pontevedra	1	*			
		Ría de Vigo	2	*			
		Punta Umbría	4	*			
		Algeciras Bay	2A, 2B, 3B, 4B	*			
		Almuñécar	1, 2, 3, 4	*			
		Garrucha	1, 2, 3	*			
		Denia	1, 2	*			
		Blanes	3, 4	*			
	P. dolichurus	Ría de Ares		Sánchez *et al.* 2011			
	P. zelinkaei	Algeciras Bay	4A	*	X		X
		Blanes	5, 6	*			
	P. sp.1	Ría de Ares	3B	*	X	X	X
		Ría de Ferrol	5B	*			
		Ría de La Coruña	2B	*			
		Ría de Vigo	2	*			
		Algeciras Bay	4A	*			
		Almuñécar	3	*			
	P. sp.2	Gulf of Cádiz	2	*		X	X
	P. sp.3	Blanes	3, 4	*	X		X

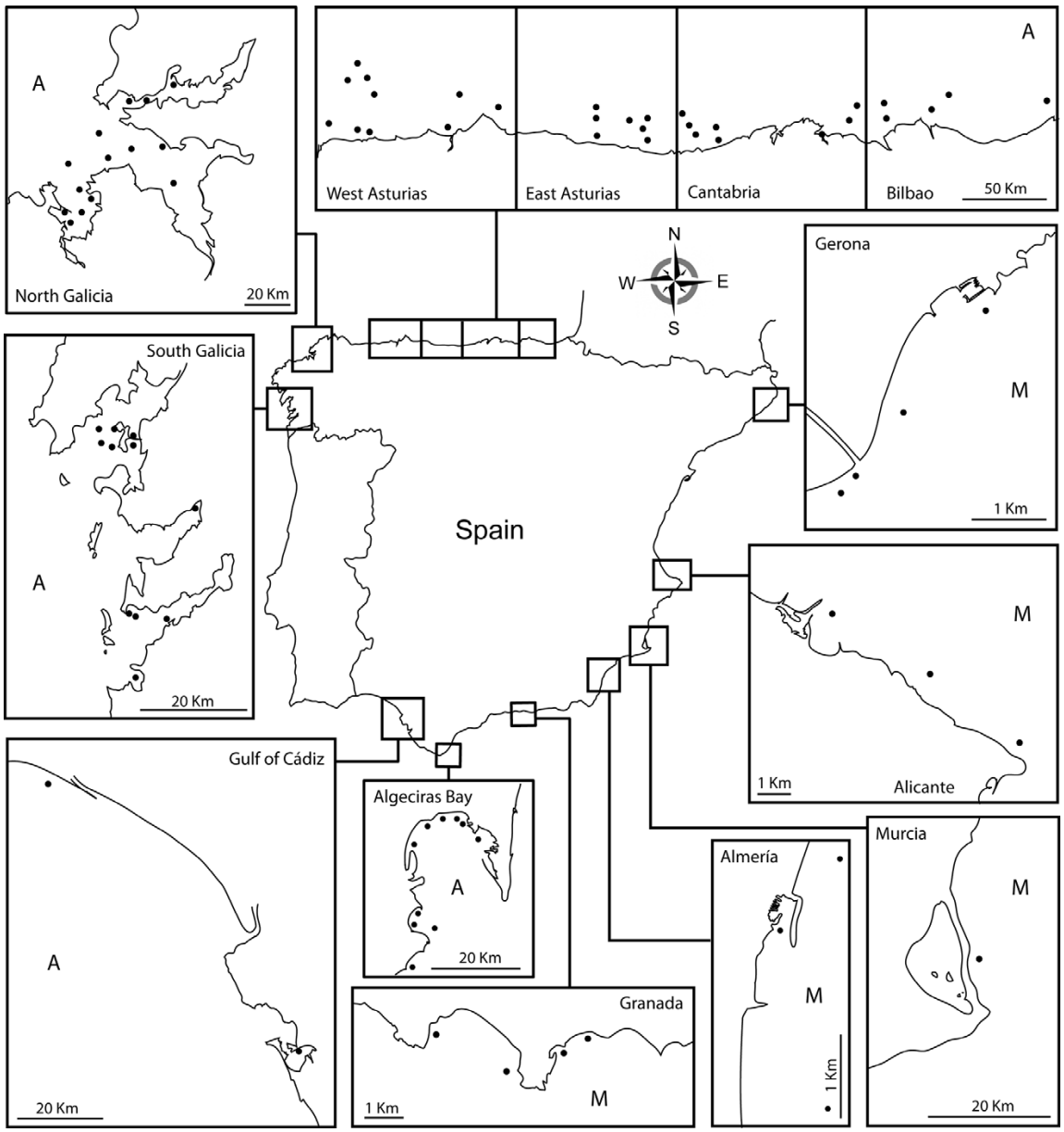

FIGURE 1. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs along the Iberian Peninsula. Abbreviations: M, Mediterranean Sea; A, Atlantic Sea.

Discussion

Diversity. Eighteen cyclorhagid species in eight different genera (*Antygomonas*, *Campyloderes*, *Centroderes*, *Condyloderes*, *Dracoderes*, *Echinoderes*, *Meristoderes* and *Semnoderes*) were recorded (Herranz *et al.* 2012; Sørensen *et al.* 2012a) (Table 2); five of these genera represent new range records for the Iberian fauna (Table 2). Most of the cyclorhagid genera reported here were represented by a single species only. Exceptions are *Antygomonas*, with two species (one undescribed), and *Echinoderes*, with ten species (two of them undescribed). Eleven homalorhagid species in three genera were recorded. These include one each of *Kinorhynchus* (a genus new to the Iberian fauna) and *Paracentrophyes* (Sørensen *et al.* 2010a) and nine *Pycnophyes* (three undescribed) (Sánchez *et al.* 2011) (Table 2). The most diverse genus in Iberian waters is *Echinoderes*; not surprising since this is the most species-rich genus in the phylum.

This study has revealed high kinorhynch diversity along the Iberian coast, comprising half of the 21 known genera in the phylum. Both Atlantic and Mediterranean coasts show localities with high diversity of genera and species (Fig. 6). Although the number of species in Atlantic areas is higher than in Mediterranean ones, an ANOVA test (p=0.89) shows that there are no significant difference in taxon diversity. This conclusion should be interpreted with caution, for the sampling effort is still biased towards Atlantic localities. We would expect that future sampling in the Mediterranean will balance these results and show a different picture.

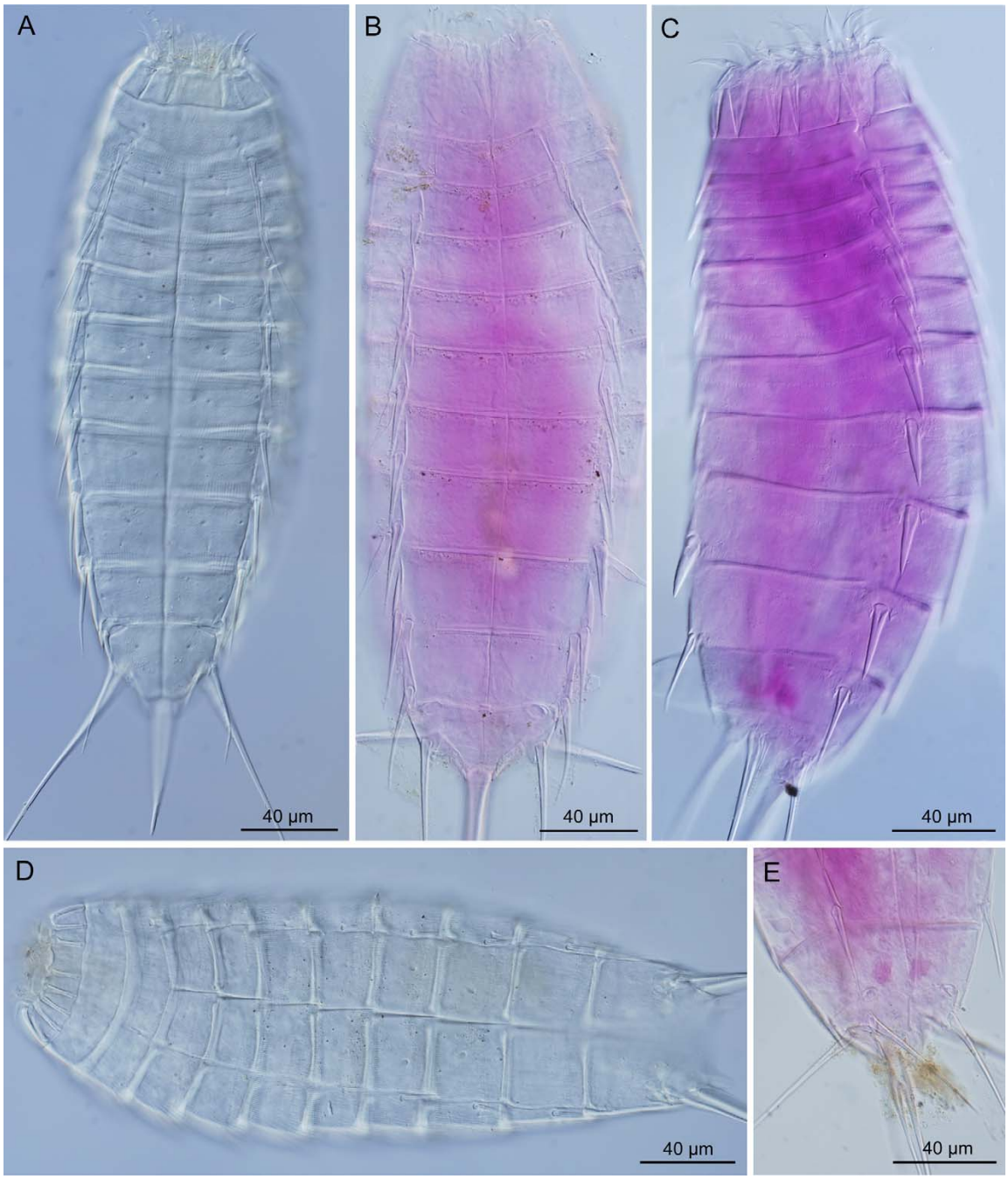

FIGURE 2. Light micrographs. **A,** *Campyloderes* cf. *vanhoeffeni*, ventral view. **B,** *Semnoderes armiger*, female, ventral view. **C,** *Centroderes spinosus*, female, dorsal view. **D,** *Echinoderes dujardinii*, male, ventral view. **E,** Ventral detail of segments 9–11 in a female of *Centroderes spinosus*.

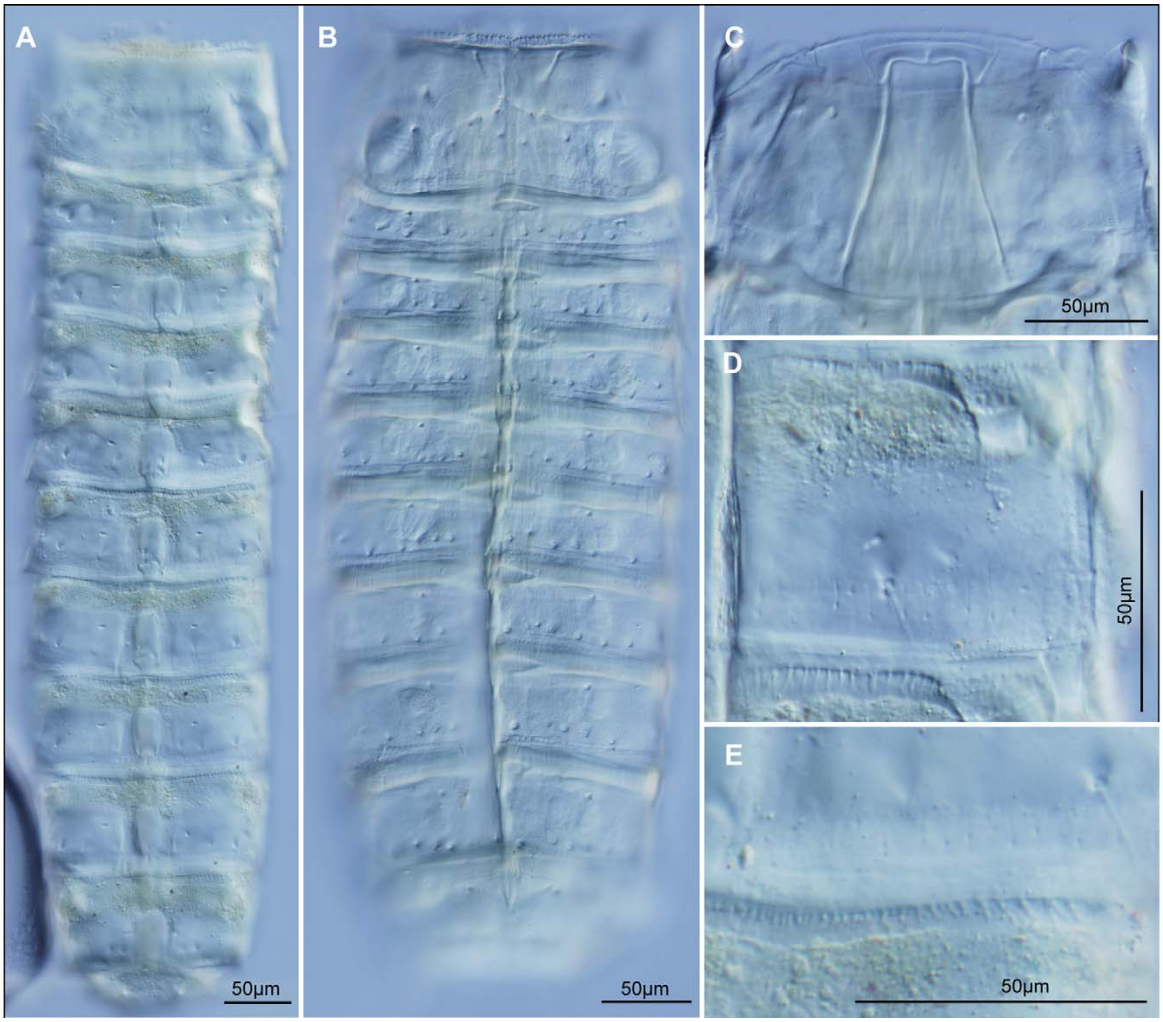

FIGURE 3. Light micrographs. **A,** *Pycnophyes zelinkaei*, male, dorsal view. **B,** *Pycnophyes carinatus*, dorsal view. **C,** *P. carinatus*, ventral view, showing placids and the shape of the midsternal and episternal plates. **D,** *P. zelinkaei*, ventral view, showing the serrated pectinate fringe from segment 8. **E,** *P. zelinkaei*, dorsal view, showing the serrated pectinate fringe from segment 8.

Geographic distribution. Knowledge about kinorhynch distribution generally is scarce. Available published data refer only to species found in particular sampling localities chosen independently. Extensive areas and/or long coastlines have only infrequently been subject to a systematic sampling program. Our data compensate for this deficiency.

Overall, the 29 species reported herein are distributed as follows (see Fig. 5 for localies):

(1) Five exclusively Mediterranean species: *Antygomonas incomitata*, *Meristoderes macracanthus*, *Pycnophyes carinatus*, *P. communis* and *Pycnophyes* sp. 3;

(2) 11 species found only in Atlantic waters: *Antygomonas* sp. 1, *Condyloderes* sp. 1, *Dracoderes gallaicus*, *Echinoderes kristenseni*, *E. neospinosus*, *E. parrai*, *E. worthingi*, *Kinorhunchus* sp. 1, *Paracentrophyes quadridentatus*, *P. dolichurus* and *Pycnophyes* sp. 2

(3) 13 species present in both seas: *Campyloderes* cf. *vanhoeffeni*, *Centroderes spinosus, E. cantabricus*, *E. dujardinii*, *E. hispanicus*, *E. isabelae*, *Echinoderes* sp. 1, *Echinoderes* sp. 2, *Semnoderes armiger*, *P. aulacodes*, *P. dentatus*, *Pycnophyes* sp. 1 and *Pycnophyes zelinkaei.*

It is tempting to think of the Strait of Gibraltar as a kind of natural limit between both seas, marking different environmental conditions that could be reflected by the species' distributions. However, it is well known that

Atlantic water enters the Mediterranean through a strong surface current, and hence, the influence of Atlantic waters and hence Atlantic species is remarkable in our samples. Therefore, the Strait of Gibraltar cannot be considered as a strong biogeographical limit in regard to kinorhynch distribution.

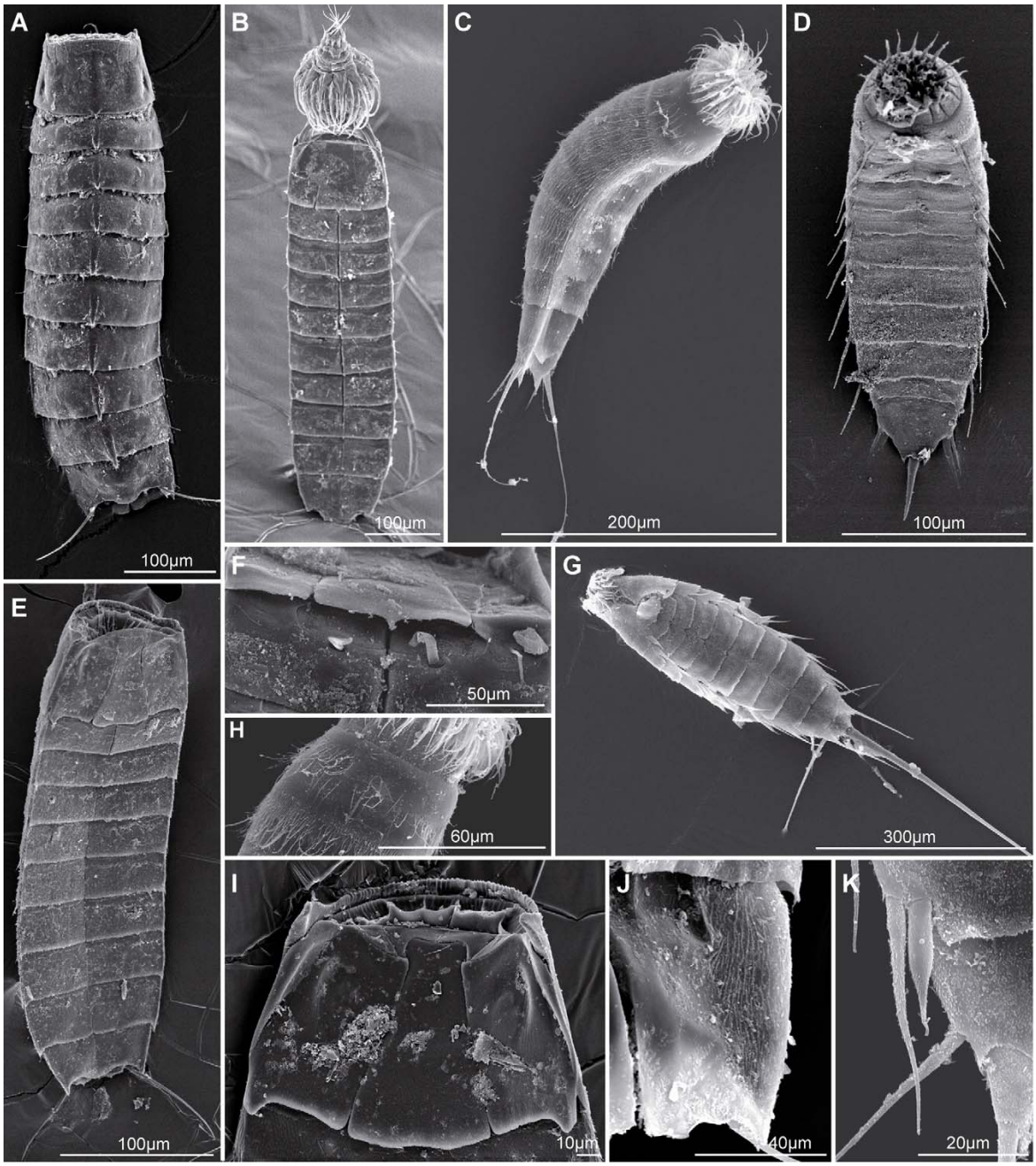

FIGURE 4. Scanning electron micrographs. **A,** dorsal view of *Pycnophyes dentatus*. **B,** ventral view of *Pycnophyes aulacodes*. **C,** lateroventral view of *Echinoderes cantabricus*. **D,** Ventral view of *Campyloderes* cf. *vanhoeffeni*. **E,** ventral view of *Pycnophyes carinatus*. **F,** *P. aulacodes*, male, ventral view; midsternal plate with a midventral pointed projection. **G,** ventral view of *Semnoderes armiger*. **H,** *E. cantabricus*, dorsal view; midlateral tube on segment 1. **I,** *P. carinatus*, female, ventral view; placids and shape of the midsternal and episternal plates. **J,** *P. dentatus*, ventral view; cuticular structures on segment 10. **K,** *S. armiger*, ventral view; detail of cuspidate and acicular spines from segment 9.

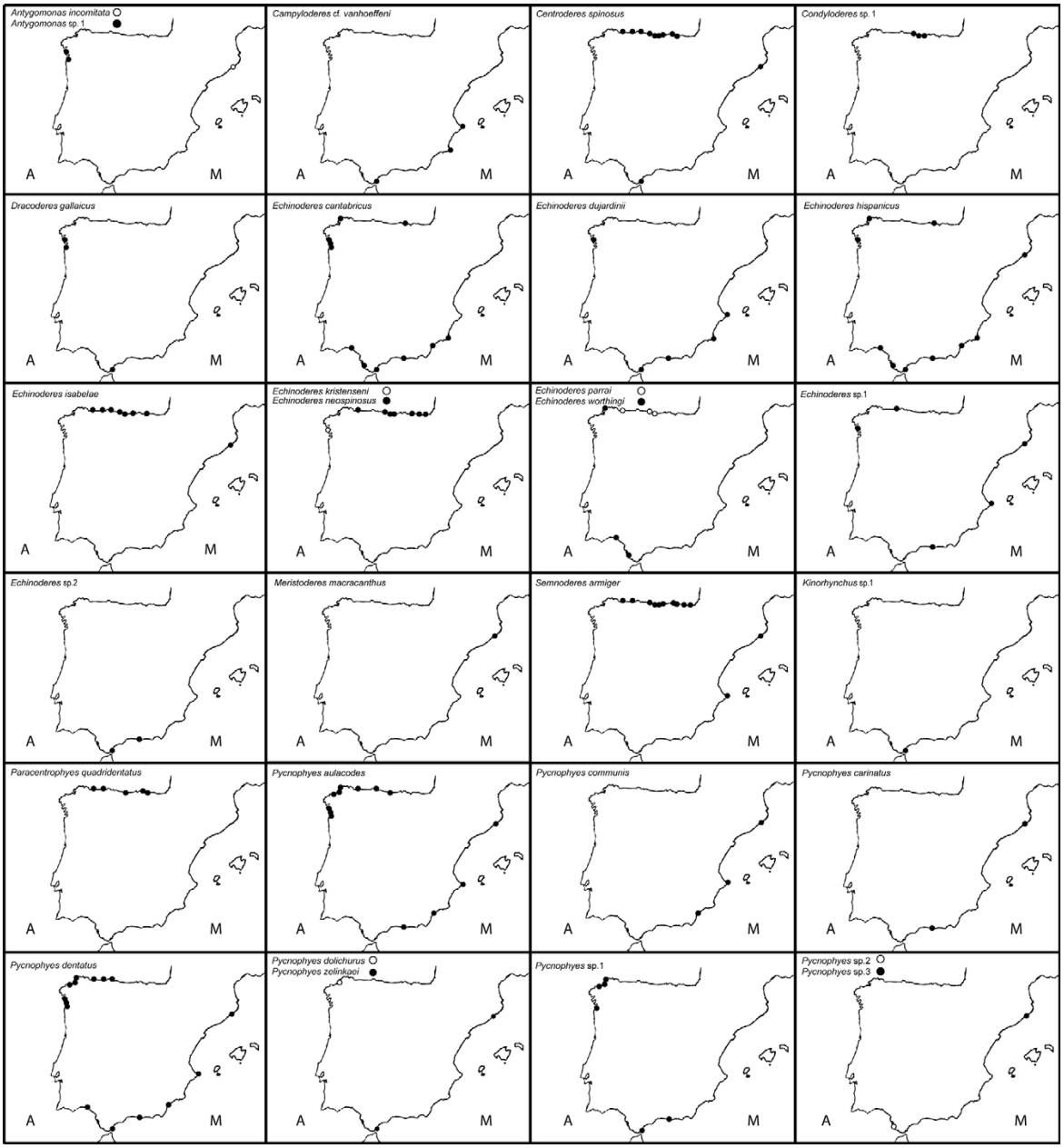

FIGURE 5. Maps showing the distribution of kinorhynch species along the Iberian coastline. Abbreviations: A, Atlantic Sea. M, Mediterranean Sea.

Our extensive sampling resulted in records of several species that were previously known only from single sites along the Iberian coast (Fig. 5, Table 2), viz. *Echinoderes cantabricus*, *E. dujardinii*, *E. hispanicus* and *E. isabelae*. *Echinoderes cantabricus* and *E. hispanicus* were described from a Cantabric locality (Atlantic) by Pardos *et al.* (1998) and have otherwise not been recorded since their discovery. Results of samplings compiled in the present study have revealed that both species are present also in Mediterranean and southern Atlantic localities (Fig. 5, Table 2). *Echinoderes dujardinii* was doubtfully reported from the Balearic Islands by Pagenstecher (1875) but recently found in Almuñécar (Granada) by Sánchez-Tocino *et al*. (2011). This species has now been found at several other Mediterranean localities and also along the Atlantic coast (Ría de Arosa, South Galicia) (see Fig. 5 and Table 2). *Echinoderes isabelae*, described from Cantabric localities (Atlantic) (GªOrdóñez *et al.* 2008), is reported

herein from Mediterranean waters (Fig. 5, Table 2). The present records suggest that the geographic range of these four species of *Echinoderes* could be even wider, at least in both east Atlantic and west Mediterranean waters.

Several species showed a wide distribution along the Iberian coast (see Fig. 5). *Pycnophyes dentatus*, a new record for the Iberian fauna, appears surprisingly to be a ubiquitous species, appearing in nearly all sampled localities (Fig. 5, Table 2). Similarly, *Pycnophyes aulacodes* was found at all Galician localities (NW), Garrucha (SE), Denia (E) and Blanes (NE) (Sánchez *et al*. 2011) and now also in Almuñécar (S) (Fig. 5 and Table 2). We interpret the absence of this species from the Gulf of Cádiz to be a matter of poor sampling, and suggest that it will turn up in the future. As stated above, *Echinoderes cantabricus*, *E. hispanicus* and *E. dujardinii* show a wide distribution along both Atlantic and Mediterranean coasts. In addition, a still-undescribed species in the same genus, *Echinoderes* sp. 1, appeared on both sides of the peninsula also, although it was recorded from fewer localities (Fig. 5 and Table 2).

The present study also shows species with an apparently more restricted distribution, sometimes found in a single or very few localities (Fig. 5 and Table 2). Only one of them is a known species and four of them are new to science and undescribed, distributed among the following genera: *Echinoderes* sp. 2, *Kinorhynchus* sp. 1, *Pycnophyes dolichurus*, *Pycnophyes* sp. 2 and *Pycnophyes* sp. 3 (Fig. 5 and Table 2). The available ecological data (see below) are not conclusive enough to affirm that they are indeed geographically isolated.

In addition, two different species of *Antygomonas* have been found along the Iberian coast: *A. incomitata* (a new record for the Iberian fauna) and a new undescribed species found in the Atlantic Ocean (Table 2). The genus *Antygomonas* has been reported from Trieste, Taranto Bay and Bari in the Mediterranean (*A. incomitata*) (Nebelsick 1990; Sørensen *et al*. 2009; Sørensen *et al*. 2010d) and from Roscoff (France) (*Antygomonas* sp. Müller & Schmidt-Rhaesa 2003) and Florida (*Antygomonas paulae* Sørensen, 2007) on both sides of the Atlantic. Therefore, it is not surprising to find a new record of the genus in European Atlantic waters (Fig. 5).

It is worth pointing out that *Echinoderes worthingi* and *E. kristenseni* are Atlantic species discovered from Ireland and Roscoff respectively (Higgins 1985). Our samples extend their known distribution, which is, however, still restricted to Atlantic waters (Fig. 5). Moreover, our data show a wider area of distribution for *Pycnophyes zelinkaei* through the Mediterranean Sea, as this species was recorded only from European Atlantic localities (United Kingdom and Ireland) until now (Southern 1914; McIntyre 1962). The present report of *P. carinatus* also extends its known distribution (Naples and Trieste) (Zelinka 1928), but is still restricted to Mediterranean waters. These four species are new to the Iberian fauna (Table 2).

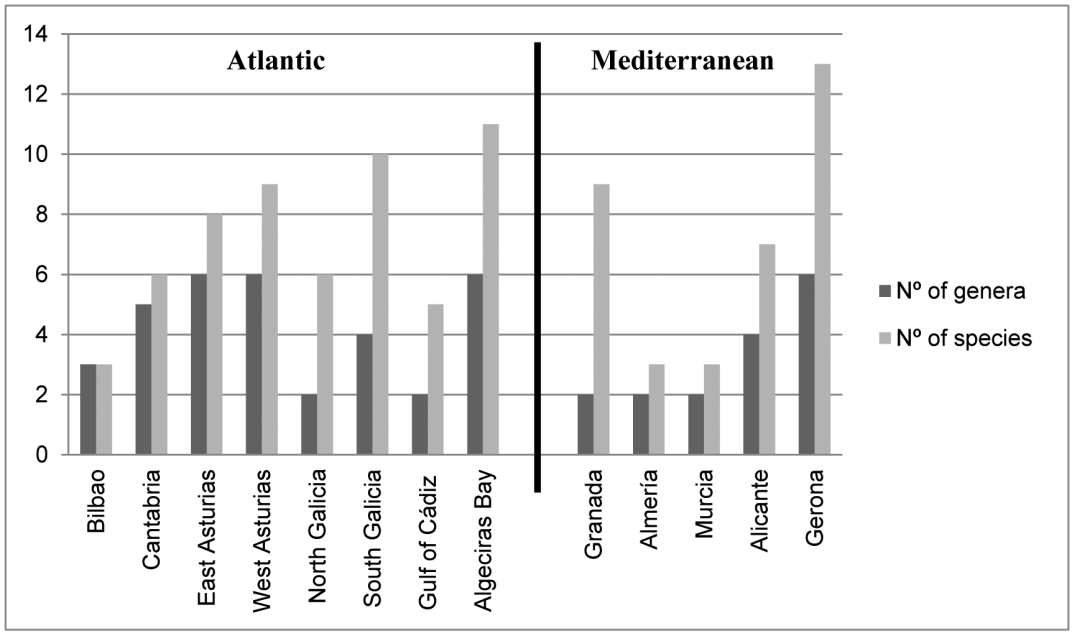

FIGURE 6. Richness of species and genera by sampling area.

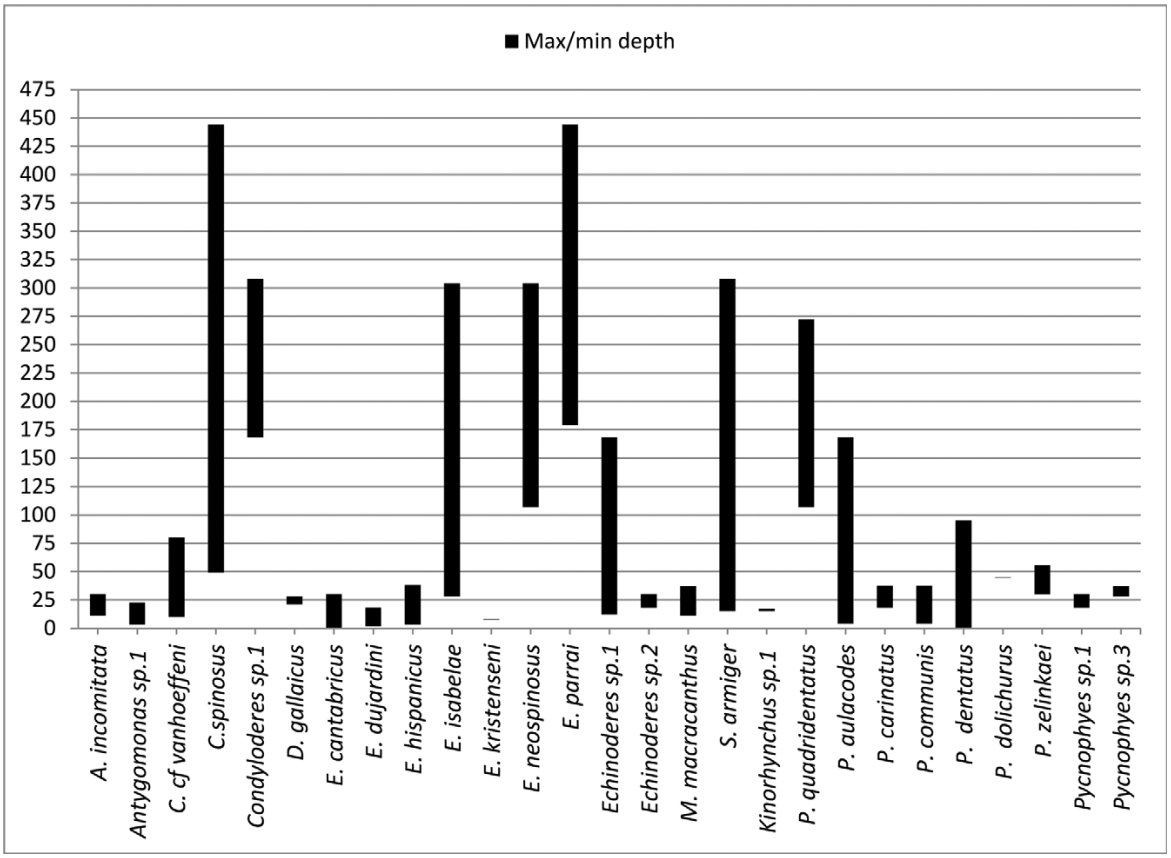

FIGURE 7. Depth records for kinorhynch species collected in this study. Data are not available for *Echinoderes worthingi* and *Pycnophyes* sp. 2.

The genera *Campyloderes* and *Condyloderes* are rare and poorly known. *Condyloderes* currently comprises five species (McIntyre 1962; Higgins 1969; Adrianov *et al.* 2002; Martorelli & Higgins 2004; Sørensen & Pardos 2008; Sørensen *et al*. 2010b), from the North Sea, Bay of Bengal, Japan, Korea and Argentina. The present finding of a yet-undescribed species is the first record of the genus from the Iberian Peninsula. *Campyloderes* cf. *vanhoeffeni* is also new for the Iberian fauna and the Mediterranean Sea (Table 2); an extensive survey of this problematic genus and its described species has been recently completed (Neuhaus & Sørensen in press).

The genera *Centroderes*, *Kinorhynchus* and *Semnoderes* are very common in European waters (Zelinka 1928; Sheremetevskij 1974; Adrianov & Malakhov 1999; Sørensen & Pardos 2008), therefore their presence along the Iberian coastline was predictable. *Centroderes spinosus* and *S. armiger* are new species records and *Kinorhynchus* sp. 1 a new genus record for the Iberian Peninsula (Table 2).

The type species of the recently described new genus *Meristoderes*, *M. macracanthus* (Herranz *et al*., 2012), has been found at only two Mediterranean localities, Blanes (type locality, northeastern Spain) and Sardinia. The distribution of the genus, with only one other known species, *M. galatheae* from the Solomon Islands (Herranz *et al*. 2012), is clearly incomplete and unworkable. The genus *Dracoderes* also mirrors such a situation; its distribution was limited to Japanese and Korean waters before Sørensen *et al.* (2012a) found a new species, *D. gallaicus* in Spain. These data clearly show that kinorhynch sampling is still very limited geographically, making our knowledge of global distribution very incomplete.

Ecology. *Depth*. Most localities reported in the present study are in relatively shallow coastal waters, less than 100 m deep. The distribution of species by depth is shown in Figure 7. Ten species of several genera appeared in deeper (>100 m) Cantabric areas (Atlantic), six of them being also reported from shallower localities; the remaining four were found only in waters deeper than 100 m, viz. *Condyloderes* sp. 1 (168–308 m), *Echinoderes parrai* (179–444 m), *E. neospinosus* (107–304 m) and *Paracentrophyes quadridentatus* (100–270 m) (Fig. 8).

Hence, it is tempting to attribute to these last four species a preference for deeper water. However, this apparent relationship may be an artifact derived from sampling design; more sampling in deeper water would be needed to confirm any such preference. These four species, apparently restricted to deep Cantabric waters, may well occur at other localities and depths (Fig. 5). In fact, the original description of *Paracentrophyes quadridentatus* is from Naples at only 15 m depth (Zelinka 1928), and species of *Condyloderes* have been reported from 40 m in India (Higgins 1969) and from 100 m and deeper in northern Europe (McIntyre 1962). The other six species taken in these deeper waters appear also in shallow waters: *Centroderes spinosus* (15–308 m), *E. isabelae* (28–304 m), *Echinoderes* sp. 1 (0–168 m), *Pycnophyes aulacodes* (4–168 m), *P. dentatus* (0–95 m) and *Semnoderes armiger* (15–308 m) (Fig. 7). All depth ranges matched with those in previous studies, except for *S. armiger* that has been reported only from shallow waters (Sheremetevskij 1974; GªOrdóñez *et al*. 2008; Sørensen & Pardos 2008; Sørensen *et al.* 2009; Sánchez *et al.* 2011). All these species have a wide range of depths and their occurrences are undoubtedly not related to depth only.

Our records of *Campyloderes* at depths ranging from 10 to 80 m fit well with the shallowest findings, although this genus has been recorded from abyssal depths down to 5000 m (Higgins 1967; Song & Chang 2001; Neuhaus 2004; Neuhaus & Sørensen in press).

Species that occurred in shallower samples (< 50 m) comprise *Antygomonas* sp. 1 (3–22.5 m), *A. incomitata* (11–30 m), *Echinoderes* sp. 2 (18–30 m), *E. cantabricus* (0–30 m), *E. dujardinii* (1.6–18 m), *E. hispanicus* (3–38 m), *E. kristenseni* (8 m), *Dracoderes gallaicus* (21–28 m), *Meristoderes macracanthus* (11–37 m), *Kinorhynchus* sp. 1 (15–17 m), *Pycnophyes* sp. 1 (18–30 m), *Pycnophyes* sp. 3 (28–37 m), *P. carinatus* (18–37.5 m), *P. communis* (4–37.5 m), *P. dolichurus* (45 m), and *P. zelinkaei* (30–55.4 m) (Fig. 7). With the exception of *P. zelinkaei*, previously recorded from samples as deep as 150 m (Southern 1914; McIntyre 1962), all data accord with the information from previous studies; therefore the occurrences of the remaining species may be depth-related (Southern 1914; McIntyre 1962; Sheremetevskij 1974; Higgins 1977; Nebelsick 1990; Pardos *et al.* 1998; Sánchez *et al.* 2011; Herranz *et al.* 2012; Sørensen *et al*. 2012a).

Sediment. As stated above under materials and methods, no standard analytical granulometric tests could be applied to all sediment samples and therefore only subjective categories could be established. Nevertheless, some sediment preferences or affinities of the kinorhynchs found in our samples can be inferred (Table 3). Several species showed no preference for any kind of sediment, appearing almost ubiquitously, viz. *Echinoderes cantabricus*, *E. dujardinii*, *E. hispanicus*, *Echinoderes* sp. 1, *Pycnophyes aulacodes*, *P. communis* and *P. dentatus.* Additional species with a similar but less-marked tendency are *E. worthingi*, *Semnoderes armiger* and *P. carinatus*.

Although appearing in several kinds of sediment, *Echinoderes* sp. 2, *Pycnophyes dolichurus*, *P. zelinkaei* and *Pycnophyes* sp. 1 are somewhat more prevalent in coarser sediments, whereas *Meristoderes macracanthus*, *E. isabelae* and *Pycnophyes* sp. 3 appeared to prefer medium grain sizes.

Finally, several species occurred exclusively in a single sediment type. The two reported species of *Antygomonas* were found only in sandy or coarse sediments, and *Dracoderes gallaicus*, *Kinorhynchus* sp. 1 and *Pycnophyes* sp. 2 were found only in fine or very fine mud.

It should be noted that the *maërl* sediment showed a surprisingly low abundance of kinorhynchs, and meiofauna in general, given that this type of substratum is usually considered as a favourable environment for marine fauna, associated with elevated species diversity.

Abundance. Although the sampling design was non-quantitative, some general observations can be made. The known patchy distributions of meiofaunal communities in a given locality (Coull 1988) can explain the high variability in specimen numbers from sample to sample. These great differences in abundance do not appear to be related to sediment preferences per se, and they are probably species-specific. For example, some species of *Pycnophyes* and *Echinoderes*, such as *P. dentatus*, *E. cantabricus* and *E. hispanicus* were always present in high numbers whereas *Campyloderes* cf. *vanhoeffeni*, *P. carinatus* and *P. zelinkaei* were represented by only one or two specimens per sample.

No definite conclusions can be deduced from the comparison between numbers of cyclorhagids and homalorhagids at every sampling station (Table 1), however more-specific studies, with quantitative approaches and methods, could reveal different patterns of patchiness between or within both groups.

TABLE 3. Relationship between species occurrence and sediment type in this study. Sediment type is arranged into eight categories by grain size, from largest (maërl) to smallest (fine mud).

Species	Maërl	Shell gravel	Coarse sand	Fine sand	Muddy sand	Sandy mud	Mud	Fine mud
Antygomonas incomitata				X				
Antygomonas sp. 1	X	X	X	X				
Campyloderes cf. *Vanhoeffeni*			X	X				
Centroderes spinosus			X		X			
Condyloderes sp. 1					X			
Dracoderes gallaicus							X	X
Echinoderes cantabricus		X	X	X		X	X	X
Echinoderes dujardinii		X	X			X	X	
Echinoderes hispanicus		X	X	X	X	X	X	X
Echinoderes isabelae				X	X			
Echinoderes kristenseni	X							
Echinoderes neospinosus					X			
Echinoderes parrai					X			
Echinoderes worthingi		X	X				X	
Echinoderes sp. 1			X	X	X		X	
Echinoderes sp. 2		X	X	X				
Meristoderes acracanthus				X	X			
Semnoderes armiger			X		X		X	
Kinorhynchus sp. 1								X
Paracentrophyes quadridentatus					X			
Pycnophyes aulacodes			X	X	X		X	
Pycnophyes carinatus			X		X		X	
Pycnophyes communis			X	X	X		X	
Pycnophyes dentatus		X	X	X	X	X	X	X
Pycnophyes dolichurus			X	X				
Pycnophyes zelinkaei		X	X	X				
Pycnophyes sp. 1		X	X	X	X			
Pycnophyes sp. 2							X	
Pycnophyes sp. 3				X	X			

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Appendix I. Additional data from collections after 2011

This Appendix gathers unpublished supplementary data from studies carried out in several localities after the publication of the first biogeographical study on the Iberian coasts (Chapter I). These data correspond to samplings in the south coast of the Iberian Peninsula and Ceuta (Figs A.I. 1, 2; Table A.I. 1) which contribute to complete and widen the biogeographic data provided in Chapter I. Additional information from newly sampled areas such as Naples (Italy) (Fig. A.I. 3, Table A.I. 2), Florida (USA) (Fig. A.I. 4, Table A.I. 3) and both Caribbean and Pacific Panama is included as well (Fig. A.I. 5, Table A.I. 4).

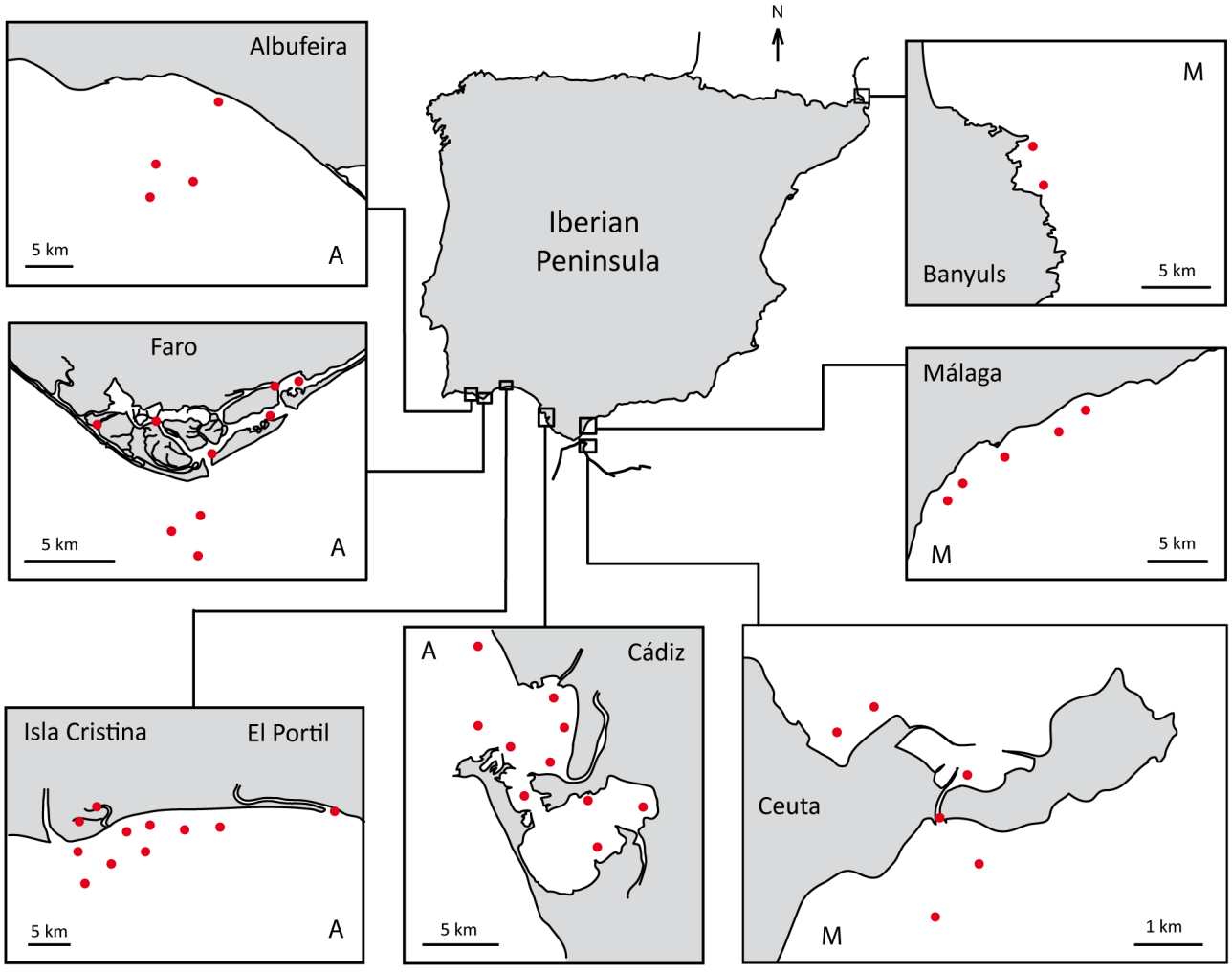

Fig. A.I. 1. Map showing new collecting areas and sampling localities (close-up in the insets) yielding Kinorhynchs along the southern coast of the Iberian Peninsula, Ceuta, and Banyuls (France).

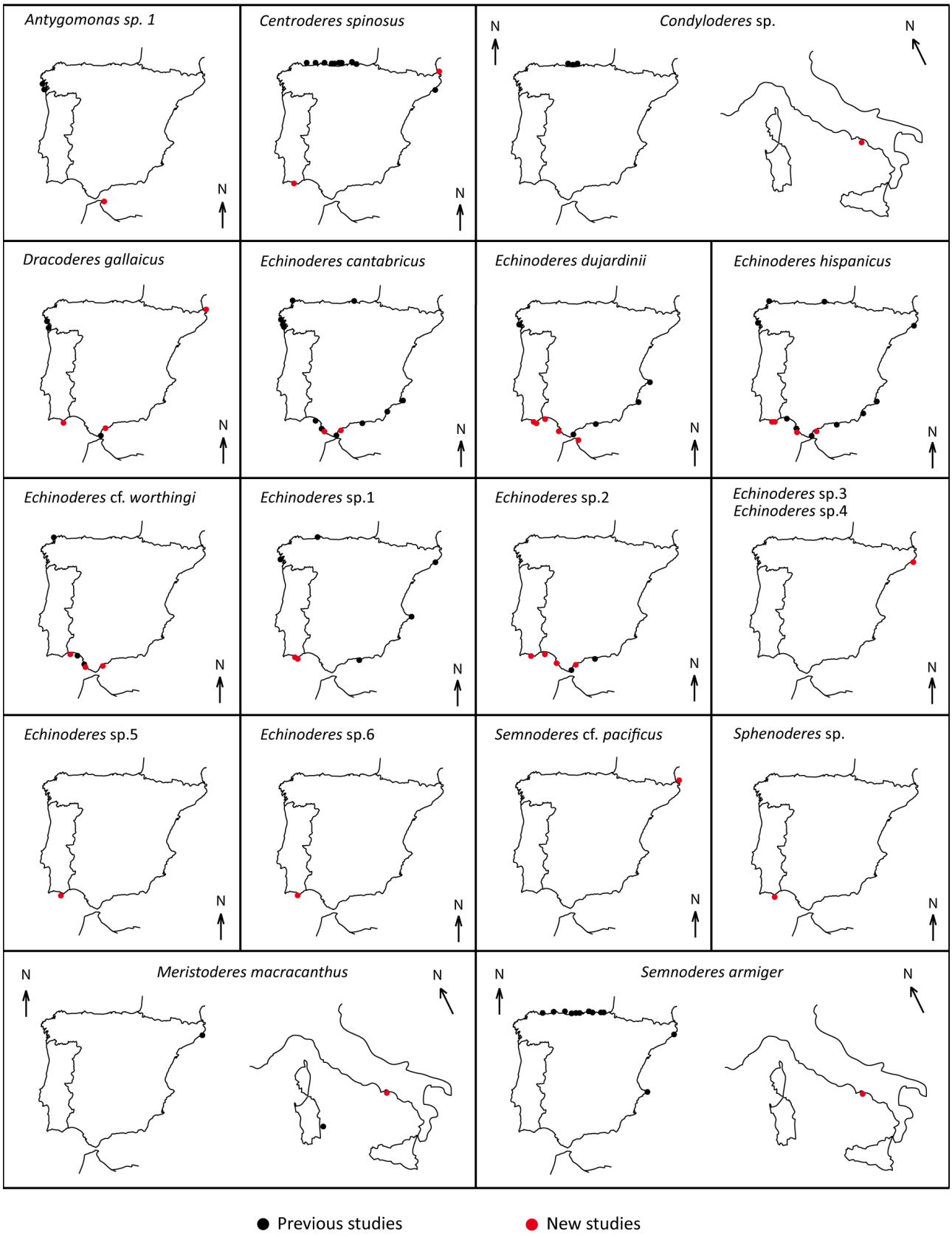

Fig. A.I. 2. Maps showing the widen distribution of species reported in the previous biogeographic study (Chapter I) but present in the new sampled areas of the Iberian Peninsula, Ceuta and Naples, including additional new species.

Table A.I. 1. Collection data and kinorhynch species from the Iberian Peninsula and Ceuta. Collectors: MH, FP, NS, RCN.

Locality	Date	Sample	Geographical coordinates	Depth (m)	Sediment	Species
Albufeira	October 28th 2013	1	36°57.664′ N 8°09.637′ W	45	Mud	*Centroderes spinosus* *Dracoderes gallaicus* *Echinoderes* sp.1 *Echinoderes hispanicus* *Echinoderes* sp.5
		2	36°59.866′ N 8°09.313′ W	35	Mud	*Dracoderes gallaicus* *Echinoderes* sp.1
		3	37°00.260′ N 8°09.266′ W	25	Mud	*Dracoderes gallaicus* *Echinoderes hispanicus*
		4	37°02.715′ N 8°06.828′ W	15	Sand	*Echinoderes* sp.1
Banyuls	September 11th 2013	1	42°29.108′ N 3°09.044′ E	35	Mud	*Echinoderes* sp.1 *Centroderes spinosus* *Dracoderes gallaicus* *Semnoderes.* cf. *pacificus*
		2	42°29.898′ N 3°08.996′ E	35	Mud	*Echinoderes* sp.1 *Centroderes spinosus*
Cádiz	November 10th 2011	1	36° 33.755′ N 6° 18.500′ W	13	Muddy sand	*Echinoderes* sp.2
		3	36° 34,117′ N 6° 15.141′ W	7	Sandy mud	*Echinoderes dujardinii*
		4	36° 32,761′ N 6° 16.268′ W	11	Coarse sand	*Echinoderes hispanicus*
		5	36° 32.310′ N 6° 14.245′ W	4	Muddy sand	*Echinoderes* cf. *worthingi*
		6	36° 31.930′ N 6°12.960′ W	4	Shell gravel	*Echinoderes dujardinii* *Echinoderes hispanicus* *Echinoderes* cf. *worthingi*
	November 11th 2011	1	36° 31.124′ N 6° 15.692′ W	11	Muddy sand	*Echinoderes dujardinii* *Echinoderes hispanicus* *Echinoderes* cf. *worthingi* *Echinoderes* sp.2
		2	36° 28.304′ N 6° 10.936′ W	4	Shell gravel	*Echinoderes cantabricus* *Echinoderes hispanicus*
		3	36° 29.798′ N 6° 12.871′ W	1	Mud with *Zostera* sp.	*Echinoderes cantabricus* *Echinoderes dujardinii* *Echinoderes hispanicus*
Ceuta	May 22th 2013	1	35° 52.989′ N 5° 18.929′ W	12	Coarse sand	*Antygomonas* sp.1 *Echinoderes dujardinii*
		5	35° 53.124′ N 5° 19.113′ W	3	Fine sand	*Echinoderes dujardinii*
	May 23th 2013	2	35° 54.032′ N 5° 19.796′ W	20	Rocks	*Echinoderes dujardinii*
		4	35° 52.989′ N 5° 18.929′ W	12	Coarse sand	*Antygomonas* sp.1 *Echinoderes dujardinii*
		5	35° 53.426′ N 5° 18.997′ W	3	Sandy mud and stones	*Echinoderes dujardinii*
El Portil	May 6th 2012	1	37° 07.632′ N 6° 48.902′W	Intertidal	Stones	*Echinoderes dujardinii* *Echinoderes* cf. *worthingi*

(Continued next page)

Table A.I. 1. (Continued)

Locality	Date	Sample	Geographical coordinates	Depth (m)	Sediment	Species
Faro	December 14th 2012	1	37°00.232' N 7°57.912' W	3	Sandy mud	*Echinoderes dujardinii* *Echinoderes hispanicus*
		2	36°59.311' N 7°53.260' W	7	Mud with shell gravel	*Echinoderes dujardinii* *Echinoderes hispanicus*
		3	37°00.114' N 7°54.989' W	10	Mud with shell gravel	*Echinoderes dujardinii* *Echinoderes hispanicus*
	October 21st 2013	0	37° 00' N 7° 88' W	Intertidal	Mud with *Zostera* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
		1	36°54.395' N 7°53.970' W	96	Mud	*Antygomonas* sp.1 *Dracoderes gallaicus* *Echinoderes* sp.1 *Echinoderes* sp.5 *Sphenoderes* sp.
		2	36°54.454' N 7°52.214' W	100	Mud	*Dracoderes gallaicus* *Echinoderes* sp. 1 *Echinoderes* sp. 5 *Echinoderes* sp. 6
		3	36°56.863' N 7°52.525' W	66	Mud	*Dracoderes gallaicus*
		4	36°57.125' N 7°52.391' W	44	Mud	*Echinoderes hispanicus*
	October 26th 2013	1	37°01.695N 7°49.014W	3	Mud with *Zostera* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
		2	37°02.199N 7°47.592W	Intertidal	Mud with *Zostera* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
		3	37°01.743N 7°47.924W	4	Mud with *Zostera* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
		4	37°00.180N 7°49.339W	2	Mud and *Ulva* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
		5	37°59.573N 7°52.745W	3	Mud with *Zostera* sp.	*Echinoderes dujardinii* *Echinoderes hispanicus*
Isla Cristina	April 11th 2011	1	37° 12.320' N 7° 20.534' W	4	Shell gravel	*Echinoderes dujardinii* *Echinoderes* sp.2
		2	37° 11.940' N 7° 21.236' W	2	Mud with shells	*Echinoderes dujardinii*
	April 12th 2011	1	37° 12.621' N 7° 20.268' W	2	Green mud	*Echinoderes dujardinii*
		2	37° 08.896' N 7° 27.916' W	9	Coarse sand	*Echinoderes hispanicus*
		3	37° 08.639' N 7° 21.176' W	12	Fine sand	*Echinoderes hispanicus*
Málaga	May 2nd 2012	1	36° 25.150' N 5° 09.830' W	35	Sandy mud	*Echinoderes* sp.2
		2	36° 26.235' N 5°04.740' W	20	Muddy sand	*Echinoderes hispanicus* *Echinoderes* sp.2
		3	36° 24.139' N 5° 09.497' W	35	Sandy mud	*Echinoderes cantabricus* *Echinoderes hispanicus* *Echinoderes* sp.2

(Continued next page)

		4	36° 24.139’ N 5° 09.497’ W	36	Sandy mud	*Echinoderes cantabricus* *Echinoderes* sp.2 *Dracoderes gallaicus*
		5	36°23.452’ N 5° 10.796’ W	36	Mud	*Echinoderes cantabricus* *Echinoderes* sp.2 *Dracoderes gallaicus*

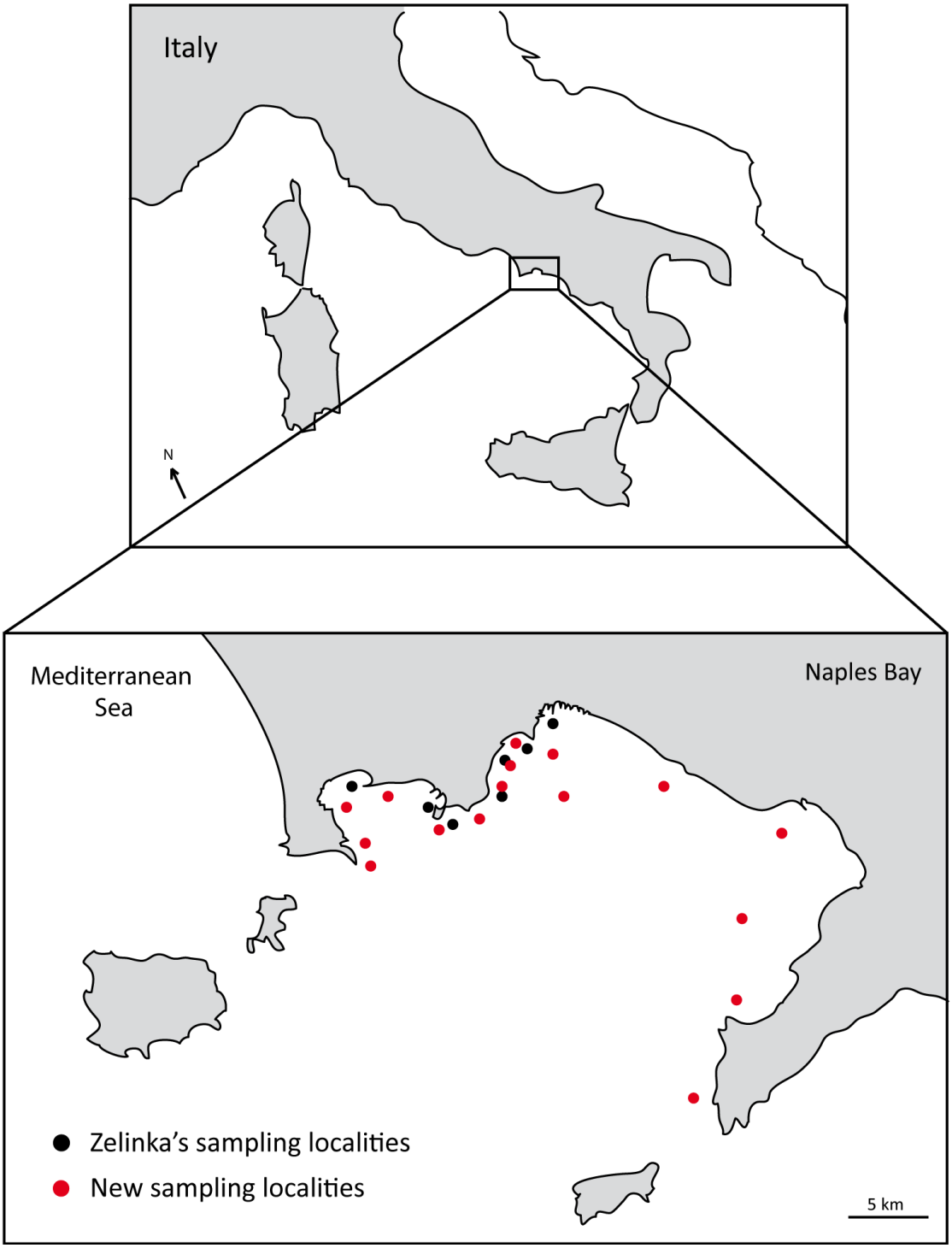

Fig. A.I. 3. Map showing sampling localities in Naples coasts.

Table A.I. 2. Collection data and kinorhynch species from Naples coasts. *Echinoderes* sp. refers to specimens not identified to the species level yet. Collectors: MH, FP, NS.

Locality	Date	Sample	Geographical coordinates	Depth (m)	Sediment	Species
Bay of Naples	September 4th 2013	1	40° 46.72' N 14° 06.79' E	37-53	Muddy Sand	No cyclorhagids
		2	40° 47.39' N 14° 05.71' E	30-52	Mud	*Echinoderes* sp.
		3	40° 48.37' N 14° 05.12' E	30-40	Mud	*Echinoderes* sp.
		4	40° 48.49' N 14° 07.85' E	40-50	Sandy mud	No cyclorhagids
		5	40° 47.15' N 14° 09.90' E	40-51	Coarse sand	No cyclorhagids
		6	40° 47.36' N 14° 11.83' E	20-18	Medium sand	*Meristoderes macracanthus*
		7	40° 48.27' N 14° 12.58' E	12-8	Fine sand	*Echinoderes* sp. *Meristoderes macracanthus*
	September 5th 2013	1	40° 48.98' N 14°13.12' E	30-29	Mud	*Echinoderes* sp. 7 *Echinoderes* sp. 10
		2	40° 49.76' N 14° 14.19' E	9-8	Muddy sand	*Echinoderes* sp. 10
		3	40° 49.51' N 14° 14.28' E	30-24	Sandy mud	*Echinoderes* sp.
		4	40° 48.77' N 14° 15,36' E	22-26	Muddy sand	No cyclorhagids
		5	40° 47.16' N 14° 14.91' E	99-98	Mud	*Echinoderes* *Semnoderes armiger*
	September 6th 2013	1	40° 36.05' N 14° 19.04' E	56-65	Muddy sand	*Echinoderes* sp.9 *Semnoderes armiger*
		2	40° 38.68' N 14° 21.78' E	99-100	Mud	*Echinoderes* sp. 7 *Echinoderes* sp.9 *Semnoderes armiger*
		3	40° 42.23' N 14° 21.71' E	117	Mud	*Condyloderes* sp. *Echinoderes* sp.8 *Semnoderes armiger*
		4	40° 45.00' N 14° 22.20' E	53-41	Sandy mud	*Echinoderes* sp.
		5	40° 47.83' N 14° 19.55' E	63-58	Mud	No cyclorhagids

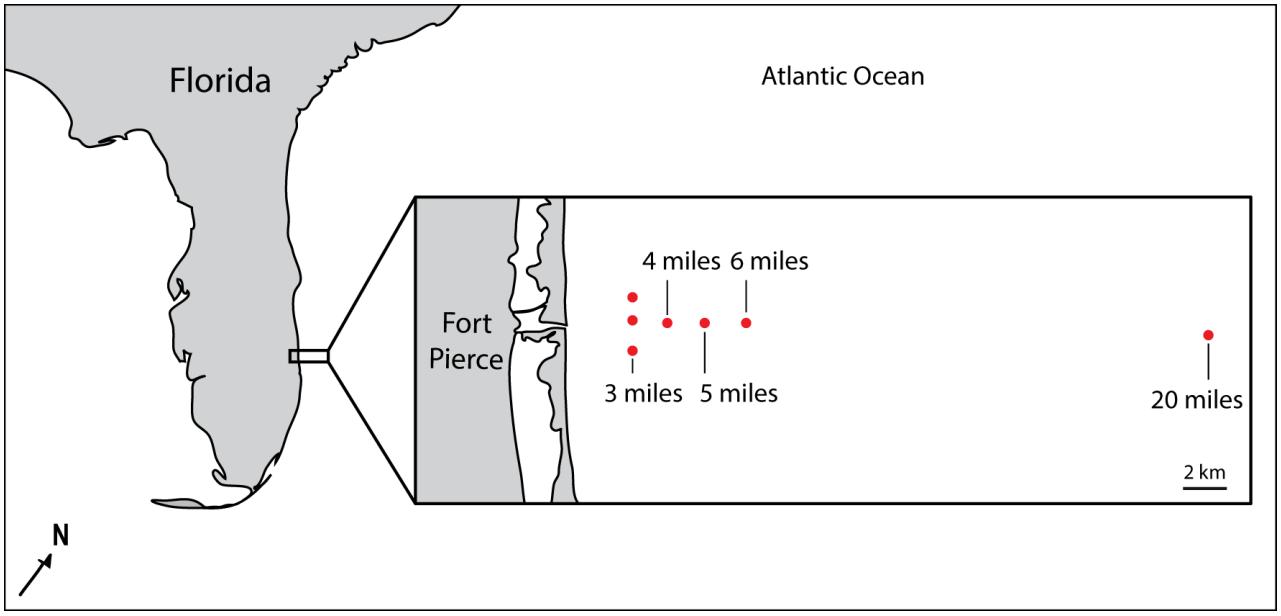

Fig. A.I. 4. Map showing sampling localities in Florida coasts.

Table A.I. 3. Collection data and kinorhynch species from the Atlantic Ocean near Fort Pierce, Florida. Collectors: MH and MJB.

Locality	Date	Sample	Geographical coordinates	Depth (m)	Sediment	Species
3 miles station	June 27th 2011 August 3rd 2011	2 3	27° 28.36' N 80° 13.69' W	10	Muddy sand	*Antygomonas paulae* *Echinoderes spinifurca* *Zelinkaderes brightae*
3 miles North station	August 3rd 2011	4	27° 29.78' N 80° 13.73' W	9	Muddy sand	*Antygomonas paulae* *Echinoderes spinifurca* *Meristoderes boylei* *Fissuroderes sorenseni* *Zelinkaderes brightae*
Capron shoals	June 27th 2011	3	27° 26,66' N 80° 13,15' N	11	Muddy sand	*Antygomonas paulae* *Echinoderes spinifurca*
4 miles station	June 6th 2011 June 27th 2011	2	27° 28,12' N 80° 12,76' W	14	Shell gravel	*Antygomonas paulae* *Echinoderes spinifurca* *Echinoderes* cf. *horni* *Zelinkaderes brightae*
5 miles station	June 6th 2011	3	27°29.96'N 80°12.67' W	15	Shell gravel	*Antygomonas paulae* *Echinoderes spinifurca* *Echinoderes* cf. *horni* *Tubulideres seminoli*
6 miles station	August 3rd 2011	4	21° 29,18' N 80° 10,98' W	15	Anfioxus sand	*Antygomonas paulae* *Echinoderes spinifurca* *Echinoderes* cf. *horni*
20 miles station	August 3rd 2011	1	27° 30,84' N 79° 54,86' W	152	Mud	*Echinoderes riceae* *Centroderes* sp. *Zelinkaderes floridensis* *Pycnophyes norenburgi*

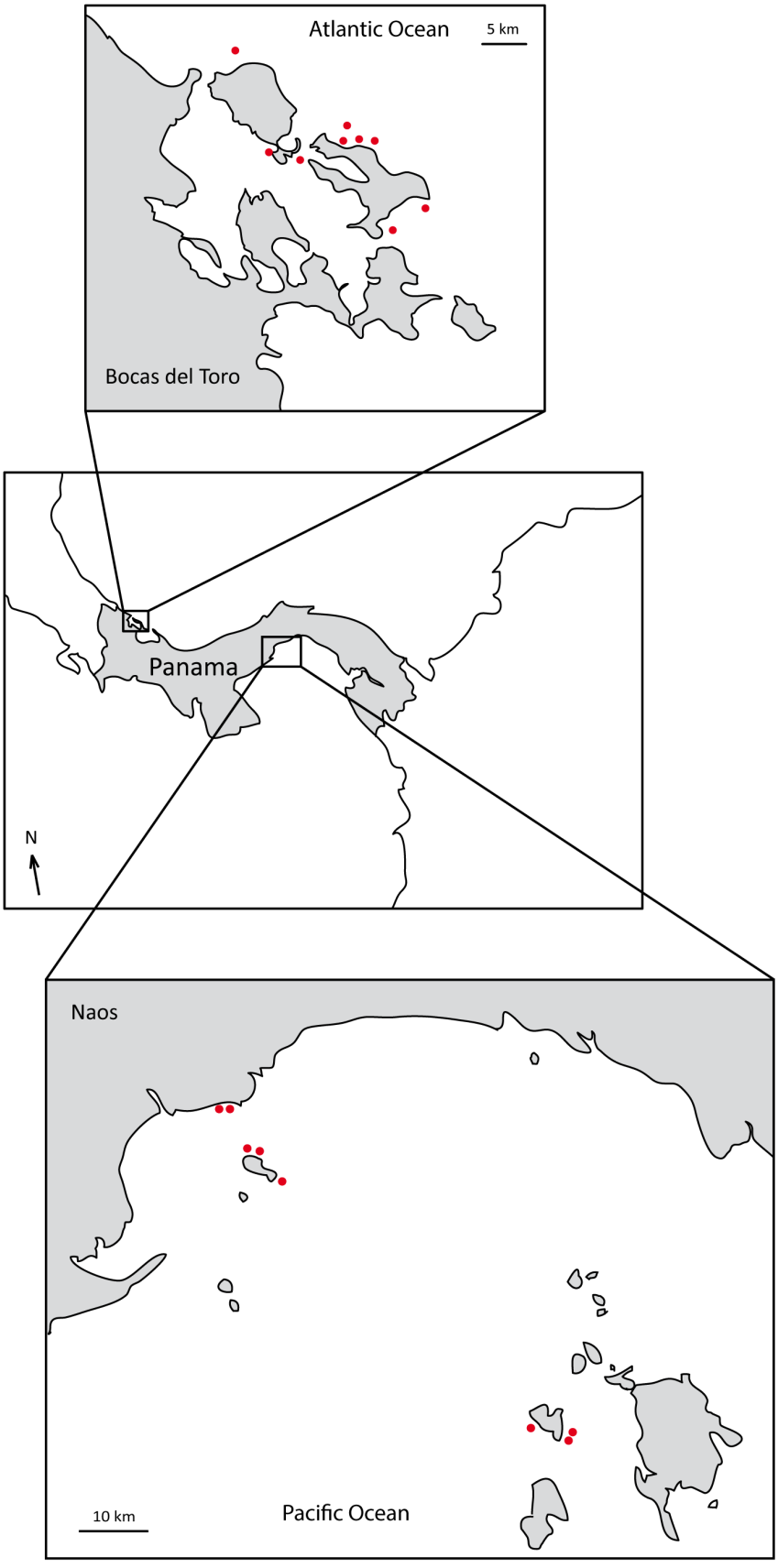

Fig. A.I. 5. Map showing sampling localities in Panama coasts.

Table A.I. 4. Collection data and kinorhynch species from Panama coasts. *Echinoderes* sp. refers to specimens not identified to the species level yet. Collector FP.

Locality	Date	Sample	Coordinates	Depth (m)	Sediment	Species
Bocas del Toro	8th June 2010	BRS 100	9° 27.5' N 82° 18.0' W	1-3	Coarse sand	*Antygomonas* cf. *paulae*
		BRS 102	9° 20.041' N 82° 13.137' W	12	Mud	*Centroderes* sp.2 *Echinoderes* sp.*12* *Echinoderes* sp.
	10th June 2010	BRS 104	9° 21.016' N 82° 10.335' W	14	Sand	*Antygomonas* cf. *paulae* *Echinoderes* sp.
	13th June 2010	BRS 106	9° 17.175' N 82° 5.065' W	n/a	n/a	*Echinoderes* sp.11
	13th June 2010	BRS 107	9° 15.126' N 82° 7.717' W	n/a	n/a	*Echinoderes* sp.11
	17th June 2010	BRS 115	9° 21.039' N 82° 10.345' W	n/a	n/a	*Antygomonas* cf. *paulae* *Echinoderes* sp.
Naos	December 2nd 2011	NAOS 3	8° 53.480' N 79° 35.724' W	Intertidal	Coarse sand	*Echinoderes* sp.
	December 06th 2011	NAOS 7	8° 53.449' N 79° 35.719' W	Intertidal	Mud	*Echinoderes* sp.
		NAOS 11	8° 23.269' N 79° 7.531' W	20	Coarse sand	*Antygomonas* cf. *paulae* *Echinoderes* sp.
		NAOS 15a	8° 23.242' N 79° 5.028' W	Intertidal	Mud	*Echinoderes* sp.
		NAOS 15b	8° 23.242' N 79° 5.028' W	Intertidal	Mud	*Echinoderes* sp.
		NAOS 21	8° 48.133´N 79° 33.309' W	2	Muddy sand	*Echinoderes* sp.
	December 9th 2011	NAOS 18	8° 48.216' N 79° 33.245' W	9	Fine sand	*Echinoderes* sp.
		NAOS 19	8° 46.938' N 79° 32.217' W	8	Medium sand	*Echinoderes* sp.

Results
Taxonomy

Chapter II

Meristoderes gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae)

Meristoderes, un nuevo género de Kinorrincos, incluyendo la descripción de dos especies nuevas y sus implicaciones en la filogenia de los echinodéridos (Kinorhyncha: Cyclorhagida, Echinoderidae)

MARÍA HERRANZ, Jonas Thormar, Jesús Benito, Nuria Sánchez, Fernando Pardos
Zoologischer Anzeiger 251: 161–179 (2012)

En este trabajo se describe *Meristoderes* un nuevo género del filo Kinorrincos, con dos nuevas especies de España y las Islas Salomón respectivamente. El nuevo género se distingue del resto por la configuración del primer y segundo segmento del tronco. El primer segmento consiste en un anillo cuticular cerrado, mientras que el segundo segmento presenta articulaciones tergoesternales incompletas y un pliegue medioventral superficial. Esta nueva configuración cuticular puede arrojar luz sobre las relaciones filogenéticas de los kinorrincos equinodéridos. *Meristoderes macracanthus* gen. et sp. nov. se ha encontrado en las costas mediterráneas españolas y se distingue por la presencia de espinas mediodorsales en los segmentos 4, 6 y 8, túbulos ventrolaterales en el segmento 2, túbulos lateroventrales en el segmento 5, espinas lateroventrales en los segmentos 6-9, túbulos laterales accesorios en el segmento 8 y un par de túbulos laterodorsales en el segmento 10. *Meristoderes galatheae* sp. nov. se ha encontrado en las Islas Salomón y se caracteriza por la presencia de una única espina mediodorsal en el segmento 4, túbulos ventrolaterales en el segmento 2, túbulos lateroventrales en el segmento 5, espinas lateroventrales en los segmentos 6-9, túbulos laterales accesorios en el segmento 8 y túbulos subdorsales en el segmento 10. Ambas especies presentan un patrón característico de perforaciones cuticulares paraventrales en los segmentos 3-9, con largos pelos bracteados que se originan en las perforaciones posteriores de los segmentos 3-7 y 3-6, respectivamente.

El género *Meristoderes* se incluye en la familia Echinoderidae Bütschli, 1876 y parece estar estrechamente relacionado con los géneros *Cephalorhyncha* Adrianov, 1999 y *Echinoderes* Claparede, 1863. En el trabajo se discute la nueva información con la intención de aclarar la filogenia interna de la familia Echinoderidae. También se estandariza la terminología de los caracteres cuticulares en la zona de solapamiento entre segmentos consecutivos.

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Meristoderes gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae)

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ABSTRACT

A new kinorhynch genus, *Meristoderes* gen. nov., and two new species from Spain and the Solomon Islands, respectively, are described. The new genus is distinguished from all other genera by the first segment consisting of a closed cuticular ring, and the second segment having partial tergosternal junctions, and a superficial midventral fold. This is a new cuticular configuration that may shed light into the phylogenetic relationships of echinoderid kinorhynchs. *Meristoderes macracanthus* gen. et sp. nov. from the Mediterranean coast of Spain is recognised by the presence of middorsal spines on segments 4, 6 and 8, ventrolateral tubules on segment 2, lateroventral tubules on segment 5, lateroventral spines on segments 6–9, lateral accessory tubules on segment 8, one pair of laterodorsal tubules on segment 10. *Meristoderes galatheae* sp. nov. from the Solomon Islands is recognized by having a middorsal spine on segment 4 only, ventrolateral tubules on segment 2, lateroventral tubules on segment 5, lateroventral spines on segments 6–9, lateral accessory tubules on segment 8 and subdorsal tubules on segment 10. Both species have a pattern of paraventral perforation site clusters on segments 3–9, with conspicuously long bracteate hairs from the posteriormost perforations sites on the segments 3–7 and 3–6, respectively.

The new genus *Meristoderes* gen. nov. is included into the family Echinoderidae Bütschli, 1876 and appears closely related with the genera *Cephalorhyncha* Adrianov, 1999 and *Echinoderes* Claparède, 1863. The new information it provides is discussed to clarify the internal phylogeny of Echinoderidae. The terminology for cuticular characters in the overlapping area between consecutive segments is also standardized.

1. Introduction

Up to now 175 valid species of kinorhynchs are known from descriptions of adult specimens; these are distributed on 20 genera (Sørensen and Pardos, 2008; Sørensen, 2008a; Sørensen and Rho, 2009; Sánchez et al., 2011; Lundbye et al., 2011; Ostmann et al., in press). However, the picture of kinorhynch taxonomy is far to be complete; five new genera have been described in the last few years (Neuhaus and Blasche, 2006; Sørensen et al., 2007; Sørensen, 2008a; Sørensen and Rho, 2009; Sørensen and Thormar, 2010), some of them still with uncertain taxonomical position and relationships. These findings, together with the information presented herein, suggest that the diversity of kinorhynch taxa is much greater than expected at all taxonomical levels.

The family Echinoderidae Bütschli, 1876 comprises four genera: *Echinoderes* Claparède, 1863, *Cephalorhyncha* Adrianov, 1999, *Fissuroderes* Neuhaus and Blasche, 2006 and *Polacanthoderes* Sørensen, 2008 (Adrianov and Malakhov, 1999; Neuhaus and Blasche, 2006; Sørensen, 2008b). The largest genus, *Echinoderes*, contains 69 valid/recognizable species to date, while the latter three contain only three, five and a single species, respectively. These four are mainly distinguished by the presence or absence of ventral divisions of the second trunk segment. In this paper we describe a new genus, *Meristoderes*, with two new species, *Meristoderes macracanthus* and *Meristoderes galatheae*. The new genus is distinguished from the others by a new cuticular configuration on segment 2, with incomplete divisions or joints.

The two new species were collected more than 15,000 km apart, from the Mediterranean coast of Spain and from the Solomon Islands in the eastern Pacific Ocean. The Mediterranean coasts have been among the most intensively investigated, not only for meiofauna in general (Cibic et al., 2009; Dinet et al., 1973; Tselepides and Lampadariou, 2004; Lampadariou et al., 2005; Lampadariou and

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Table 1
Information of the samples collected on March 24, 1999 in Blanes (Gerona), NE Spain.

Station	Locality	Coordinates	Sediment	Depth
1	Blanes	41°40.210′ N02°47.740′ E	Sand	11.6 m
2	Blanes	41°39.326′ N02°47.000′ E	Muddy sand	11 m
3	Blanes	41°38.596′ N02°46.255′ E	Midfine sand	28.4 m
4	Blanes	41°38.511′ N02°46.322′ E	Muddy sand	37.5 m

Tselepides, 2006) but also for kinorhynchs (Băcescu and Băcescu, 1956; Băcescu, 1968; Higgins, 1978; summarized in Higgins, 1983; Nebelsick, 1990; Reinhard, 1881; Sheremetevskij, 1974; Zelinka, 1928) while only a single study of kinorhynchs have been conducted in the Solomon Islands (Thormar and Sørensen, 2010).

The new genus with two new species provides us with a new complex of characters that can contribute to clarify the evolutionary relationships of echinoderid kinorhynchs. This study also presents detailed observations on the cuticular surface structures obtained with scanning electron microscopy (SEM), especially with regard to the distribution of sensory spots and glandular pores. Data from SEM examinations are only available for very few species which stresses the importance of SEM investigations whenever the circumstances allow it (Pardos et al., 1998; Sørensen, 2007) in order to gain additional and more detailed morphological information for taxonomical and phylogenetic purposes.

2. Materials and methods

Specimens of *M. macracanthus* gen. et sp. nov. from Spain were collected at four stations in the vicinity of Blanes (Gerona, NE Spain) on March 24, 1999 (Fig. 1A). Detailed data on sampling stations are summarized in Table 1. Sediment samples were taken with a Higgins meiobenthic dredge that collects the uppermost part of the sediment only. Kinorhynchs were extracted from the sediment following the bubbling technique of Higgins (Higgins and Kristensen, 1988; Neuhaus, 2003; Sørensen and Pardos, 2008), fixed in 7% formalin and stained with Rose Bengal to facilitate sorting.

Specimens of *M. galatheae* gen. et sp. nov. were collected on January 6, 2007 during a land based part of the research cruise Galathea 3 near Ghizo Island, Solomon Islands in Melanesia (Fig. 1B–D). Samples were collected by SCUBA diving at the diving locality named "Hot Spot" (08°01.781′S 156°48.850′E) at 28 m depth by collecting sediment in square plastic bottles (Kautex wide neck containers). The substrate was coral sand from beneath a coral overhang. Water temperature was 29 °C and salinity 35‰. Samples were shaken and water was decanted through a net with a 63 μm mesh width from sediment samples that were untreated. Specimens were then fixed in 4% borax-buffered formalin.

Specimens of both species were sorted using a stereo microscope. Specimens for light microscopy (LM) were dehydrated through a graded series of ethanol and transferred to glycerine prior to mounting in Fluoromount G®. The mounted specimens were examined and photographed using Olympus BX51 and Olympus BX61 microscopes with Nomarski differential interference contrast (DIC) optics. The microscopes were equipped with the cameras Olympus Colorview 1, DP20 or DP70, and for measurements the software Olympus CellˆA® or CellD̂® were used. Specimens for scanning electron microscopy (SEM) were dehydrated from formalin fixed samples through a graded series of ethanol, transferred to acetone and critical point dried. The dried specimens were mounted on aluminum stubs, sputter coated with gold or platinum–palladium, and examined with either JEOL 6400 or JEOL JSM-6335F field emission scanning electron microscopes.

The type material (holotype, allotype and paratypes) has been deposited at the Zoological Museum, Natural History Museum of Denmark, under the accession numbers ZMUC KIN-522 to ZMUC KIN-529 for *M. macracanthus* and ZMUC KIN-393 to ZMUC KIN-396 for *M. galatheae*.

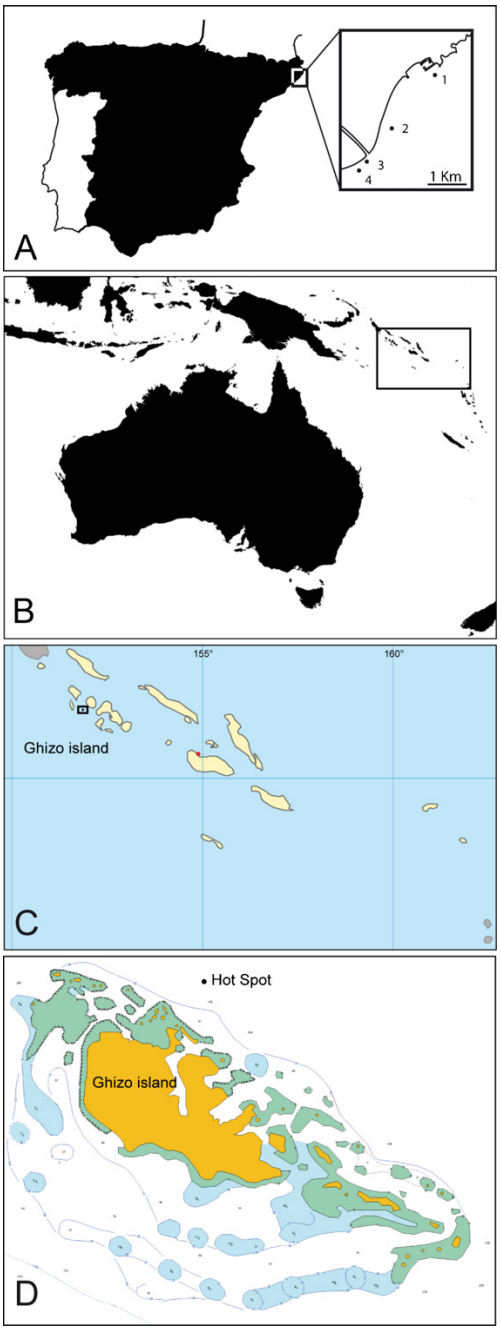

Fig. 1. Maps showing (A) the sampling locality and stations 1–4 at Blanes, NE Spain. (B) Australia and South West Pacific islands with Solomon Islands marked. (C) ArcGis map of Solomon Islands. (D) CMAP of Ghizo Island with indication of the sampling locality "Hot Spot".

2.1. Notes on terminology

Trunk segments are numbered 1–11 following Neuhaus and Higgins (2002) and Sørensen and Pardos (2008).

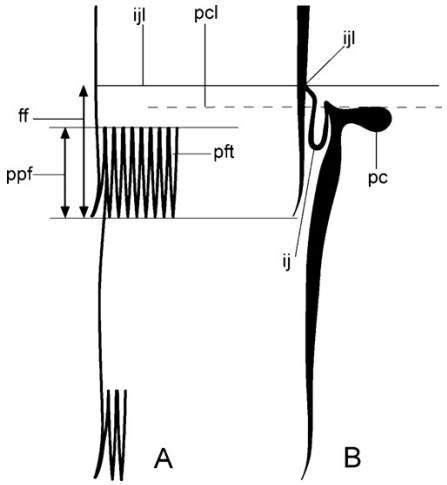

Fig. 2. Schematic illustration of two consecutive trunk segments. (A) External view. (B) Sagittal section. The free flap (ff) is the posterior edge of a segment that overlaps the following. The length of the free flap, the primary pectinate fringe (ppf) and the pectinate fringe tips (pft) are fixed and measurable. However, the position of the pachycyclus (pc) and its associated pachycyclus line (pcl) are variable and depend on the degree of contraction of the specimen. The intersegmentary joint line (ijl) marks the point of attachment of the intersegmentary joint (ij) to the inner side of the cuticle, above the pectinate fringe.

Positional terminology follows that proposed by Pardos et al. (1998) with the addition of a paraventral position (Sørensen et al., 2010) and an amended specification of the divisions between the positions proposed for the genus *Echinoderes* (Thormar and Sørensen, 2010), which can also be applied to *Meristoderes* gen. nov. Other terminology generally follows Sørensen and Pardos (2008) and Thormar and Sørensen (2010). The terms used for structures and features located at the overlapping region between consecutive segments are herein further clarified and illustrated, knowing their taxonomic relevance and their use as reference points for, e.g., measurements.

2.1.1. "Intersegmentary joint line" "free flap" and "primary pectinate fringe"

(Figs. 2A and B, 3A and B, 5F, 6F, 7A, 8A, 9C, and 10D and F)

The posterior edge of each segment overlaps the anterior edge of the next one, covering a circular band of soft cuticle that constitutes the intersegmentary joint. The anteriormost attachment site of intersegmentary joints (Gª Ordóñez et al., 2000) can usually be observed in LM as a distinct transverse line, frequently illustrated in kinorhynch descriptions but rarely reported or described in texts. We term this line the "intersegmentary joint line" or "ij-line" for short. The cuticle posterior to this line constitutes an extension which may or may not overlap the proceeding segment, depending on whether the intersegmentary joint is folded or fully stretched (Gª Ordóñez et al., 2000). We term this part of the cuticle "free flap". The posterior part of this free flap is usually constituted by a primary pectinate fringe with "fringe tips". We define the length of the primary pectinate fringe as equal to the length of its fringe tips.

2.2. Additional specimens examined

From the Natural History Museum in London (BMNH), the holotype of *Echinoderes krishnaswamyi* Higgins, 1985 and the holotype and allotype of *Echinoderes maxwelli* Omer-Cooper, 1957 were loaned for comparison. Studied type specimens from the Natural History Museum of Denmark include paratypes of *Echinoderes cantabricus* Pardos et al., 1998 (ZMUC KIN-62 to KIN-67), all type specimens of *Cephalorhyncha liticola* Sørensen, 2008 (ZMUC KIN-205 to KIN-207), all type specimens of *Echinoderes collinae* Sørensen, 2006 (ZMUC KIN-149 to KIN-155), and paratypes of *Echinoderes angustus* Higgins and Kristensen, 1988, *Echinoderes aquilonius* Higgins and Kristensen, 1988, *Echinoderes eximus* Higgins and Kristensen, 1988, and *Echinoderes peterseni* Higgins and Kristensen, 1988 (ZMUC KIN-21 to KIN-28). Original unpublished LM and SEM photos of the type specimens of *Echinoderes spinifurca* Sørensen et al., 2005 were also studied. Furthermore, several non-type specimens were studied in SEM: *E. angustus*, *E. aquilonius*, *E. eximus* all collected at their type localities in Greenland, *E. peterseni* collected in Ikka Fjord, Greenland and *E. cantabricus* collected at its type locality. LM specimens of *Echinoderes* cf. *worthingi* Southern, 1914 collected near Tjärnö Marine Laboratory, Sweden (M. V. Sørensen, personal collection), *Echinoderes* cf. *kristenseni* Higgins, 1985 collected in Denia (East coast of Spain), *Echinoderes hispanicus* and *E. cantabricus* both collected in type localities were also studied.

3. Results

Phylum	Kinorhyncha Dujardin, 1851
Order	Cyclorhagida Zelinka, 1896
Family	Echinoderidae Bütschli, 1876

3.1. Echinoderidae Bütschli, 1876

3.1.1. Diagnosis

Adults with strongly sclerotized trunk cuticle; neck with 16 placids; midventral placid broader than remaining placids. Cuticle of first trunk segment a closed ring; cuticle of second trunk segment a closed ring or with complete or incomplete midventral and lateral divisions; cuticle of trunk segments 3–10 with midventral and lateral articulations resulting in two sternal plates and one tergal plate; lateral and middorsal spines acicular, may be missing in adults of some species but not in juvenile stages; middorsal spines arranged along midline; lateral terminal spines always present; lateral terminal accessory spines absent in males; midterminal spine absent in adults; cuspidate spines absent.

Genera included: *Echinoderes* Claparède, 1863; *Cephalorhyncha* Adrianov, 1999; *Fissuroderes* Neuhaus and Blasche, 2006; *Polacanthoderes* Sørensen, 2008 and *Meristoderes* gen. nov.

3.2. Meristoderes gen. nov

3.2.1. Diagnosis

Echinoderidae with segment 2 having paired lateroventral/ventrolateral partial divisions (position corresponding to the tergosternal junctions of following segments).

3.2.2. Etymology

The genus name is derived from Greek *meristos*, meaning 'divided', making reference to the incomplete division of second trunk segment; and the suffix "*-deres*", 'neck', commonly used in generic names of cyclorhagid kinorhynchs. Gender of genus name: masculine

3.2.3. Type species

Meristoderes macracanthus
Genus *Meristoderes* gen. nov.

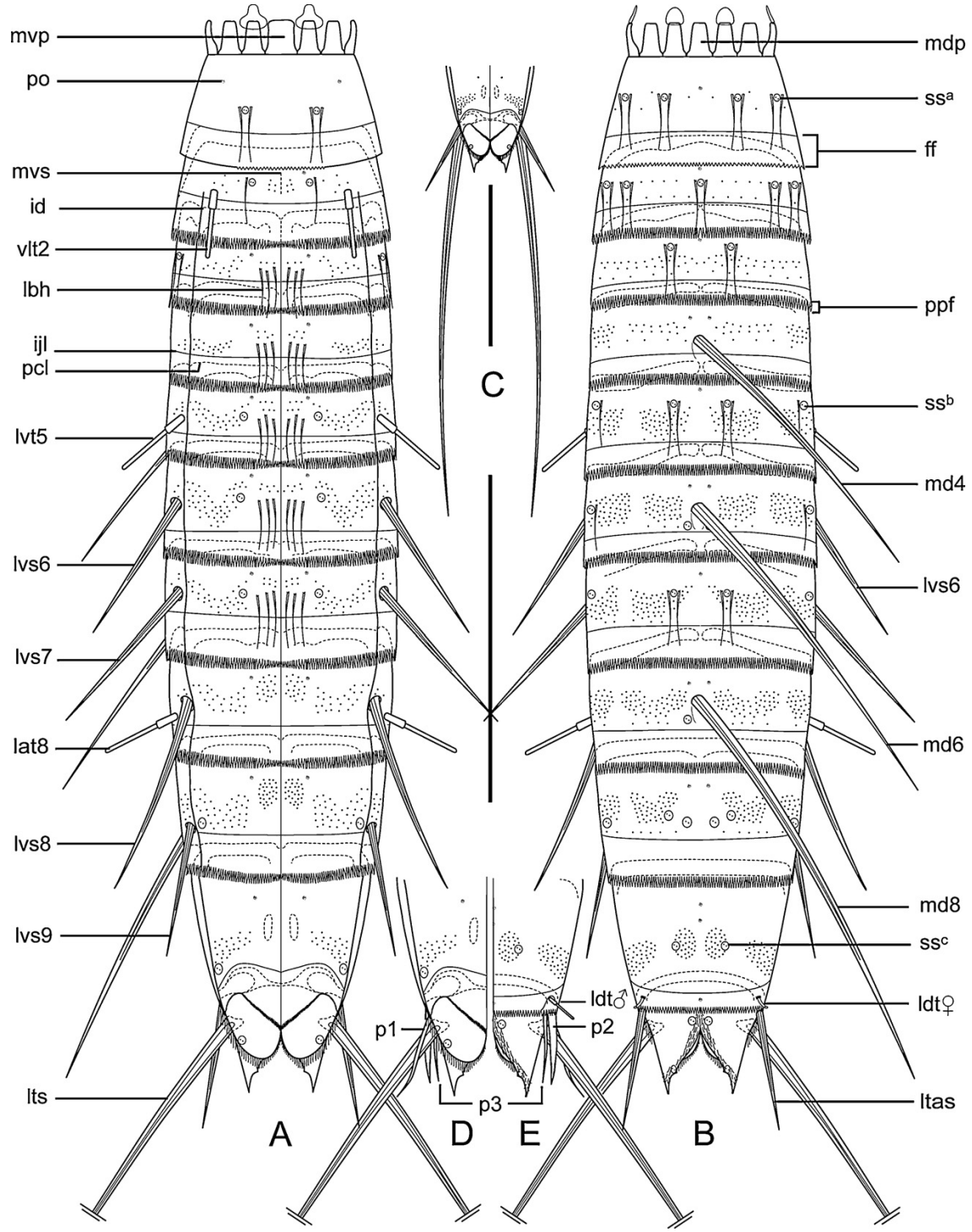

Fig. 3. *Meristoderes macracanthus* gen. et sp. nov. line art illustrations. (A) Female, ventral view. (B) Female, dorsal view. (C) Segment 11 and terminal spines, drawn in different scale to show the length of LTS. (D) Male, right part of trunk segments 10 and 11, ventral view. (E) Male, right part of trunk segments 10 and 11, dorsal view. Abbreviations: ff, free flap; id, incomplete division; ijl, intersegmentary joint-line; lat, lateral accessory tubule; lbh, long bracteate hair; ldt, laterodorsal tubule; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; mdp; middorsal placid; mvp, midventral placid; mvs, midventral split; p1-3, penile spine; pcl, pachycyclus line; ppf, primary pectinate fringe; po, pore field; ss, sensory spot, superscript refers to the sensory spot subtype (a, b, c); vlt, ventrolateral tubule. Digits after abbreviations refer to segment number.

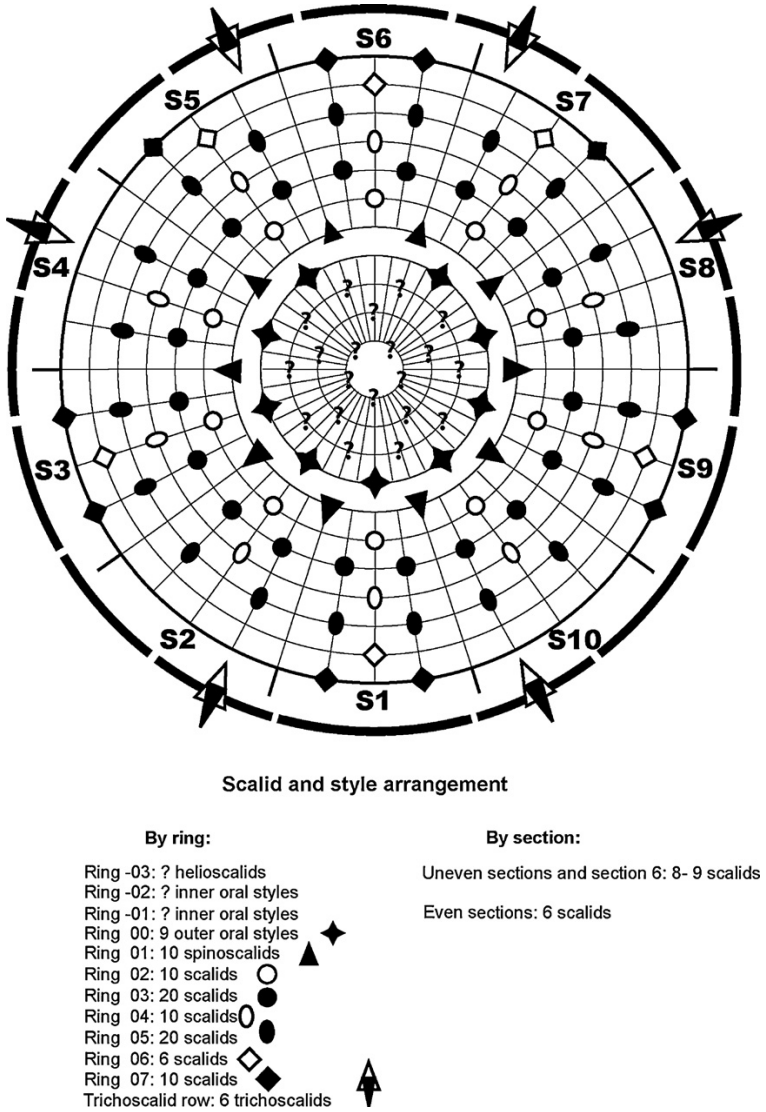

Fig. 4. Diagram of mouth cone, introvert and placids showing distribution of oral styles and scalids in *Meristoderes macracanthus* gen. et sp. nov.

3.3. *Meristoderes macracanthus* sp. nov. (Figs. 3–7 and Tables 2 and 3)

3.3.1. Diagnosis

Meristoderes with middorsal spines on segments 4, 6 and 8, increasing uniformly in length posteriorly. Ventrolateral tubules on segment 2. One pair of lateroventral tubules at segment 5; lateroventral spines on segments 6–9; lateral accessory tubules on segment 8; one pair of laterodorsal tubules on segment 10 in males and females; lateral terminal spines on segment 11 in both sexes and lateral terminal accessory spines on segment 11 in females only.

3.3.2. Etymology

The species name is derived from Greek *makro*, meaning 'long', and *akantha*, meaning 'spine', making reference to the unusual length of the cuticular spines.

3.3.3. Type locality

Blanes (Girona) NE coast of Spain, Mediterranean Sea.

Other localities: Sardinia, Mediterranean Sea (M. V. Sørensen, pers. comm., 2010).

3.3.4. Type material

A total of 40 specimens from the type locality were examined. Eight specimens were selected as type series. Holotype: adult female, mounted in Fluoromount G® (ZMUC KIN-522), collected on 24th of March 1999, off Blanes, Spain 41°39.326′N, 02°47.000′W at 11 m depth from muddy sand. Allotype: adult male, mounted in Fluoromount G® (ZMUC KIN-523), collected on 24th of March 1999, off Blanes, Spain 41°38.596′N, 02°46.255′W at 28.4 m depth from midfine sand. Paratypes: three adult females (ZMUC KIN-524 to KIN-526) and three adult males (ZMUC KIN-527 to KIN-529), all mounted in Fluoromount G®, same collecting data as for holotype. Type series is deposited at the Natural

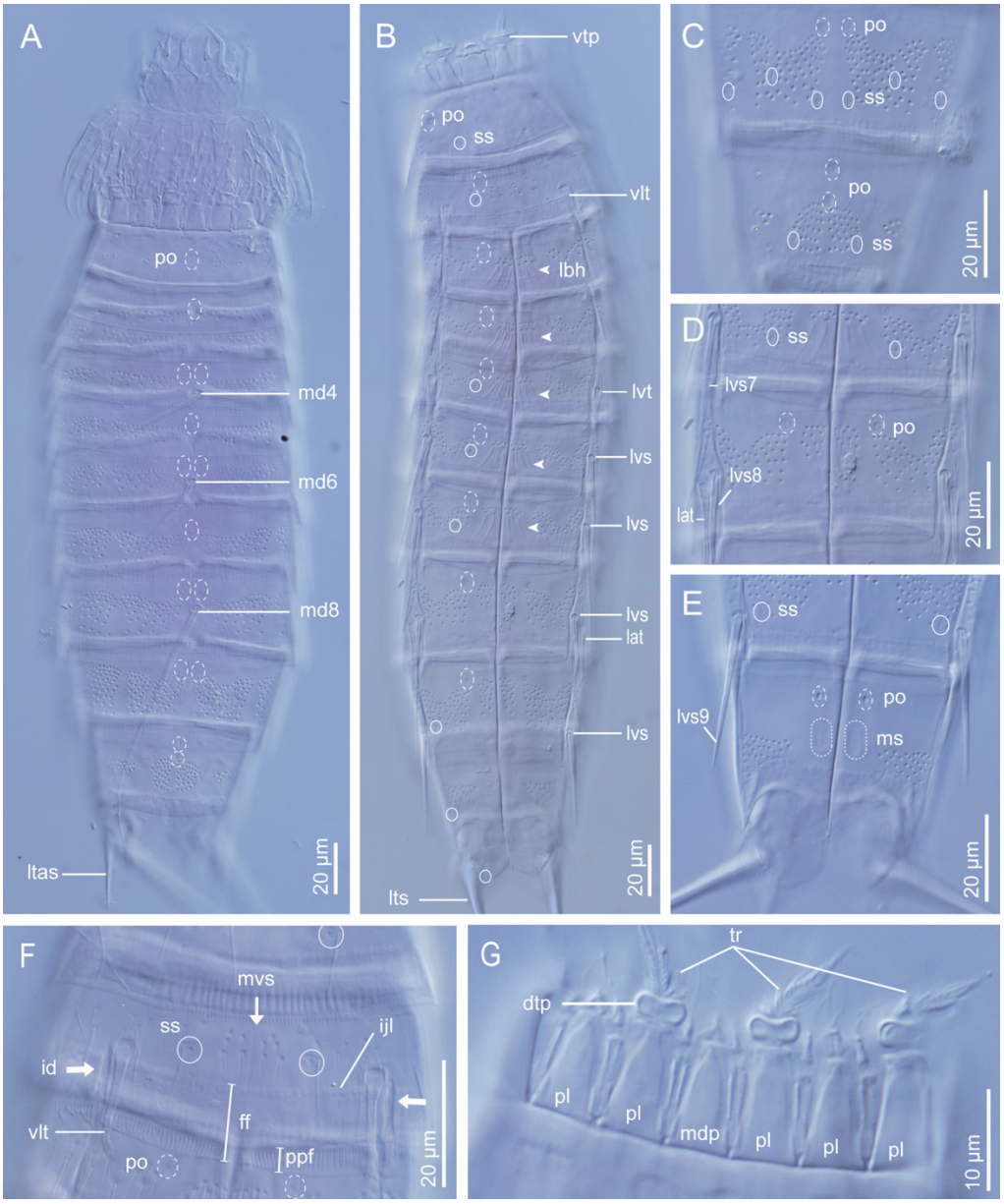

Fig. 5. *Meristoderes macracanthus* gen. et sp. nov. light microscopical photos including (A) female holotype (ZMUC KIN-522); (B and D) male allotype (ZMUC KIN-523); (C, G and E) male paratype (ZMUC KIN-XXX) and (F) female paratype (ZMUC KIN-XXX). (A) Dorsal view; (B) ventral view. Note paraventral hair clusters indicated by arrowheads. (C) Trunk segments 9–10, dorsal view. (D) Trunk segments 7–8, ventral view. (E) Trunk segments 9–11, ventral view. (F) Trunk segment 2, ventral view. (G) Neck with placids, dorsal view. White circles indicate pore fields (dotted line) and sensory spots (solid line). Abbreviations: dtp, dorsal trichoscalid plate; ff, free flap; ijl, intersegmentary joint-line; lat, lateral accessory tubule; lbh, long bracteated hairs; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; md, middorsal spine; ms, muscular scar; mdp; middorsal placid; mvs, midventral split; id, incomplete division; pl, placid; ppf, primary pectinate fringe; po, pore field; ss, sensory spot; tr, trichoscalid; vlt, ventrolateral tubule; vtp, ventral trichoscalid plate.

History Museum of Denmark. Remaining specimens are deposited in the authors' collection at the Universidad Complutense, Madrid, Spain.

3.3.5. Description

Adults with head, neck and 11 trunk segments (Figs. 3A and B, 5A and B, and 6A and B). All measurements and dimensions are given in Table 2. Table 3 shows a summary of spine, tubule and sensory spots locations.

The head consists of a retractable mouth cone and an introvert with seven rings of scalids (Figs. 4, and 6C and D). The mouth cone bears, from inner to outer, one ring of helioscalids and three rings of oral styles. The ring with the outer oral styles is referred to as ring 0; hence rings in the mouth cone will be numbered -01, -02, and -03 for the innermost ring. Helioscalids (ring -03) and inner oral styles (rings -02, -01) could not be examined in any specimen. The fourth ring (ring 0) has nine, long and pointed outer oral styles divided into two segments with fringed bases.

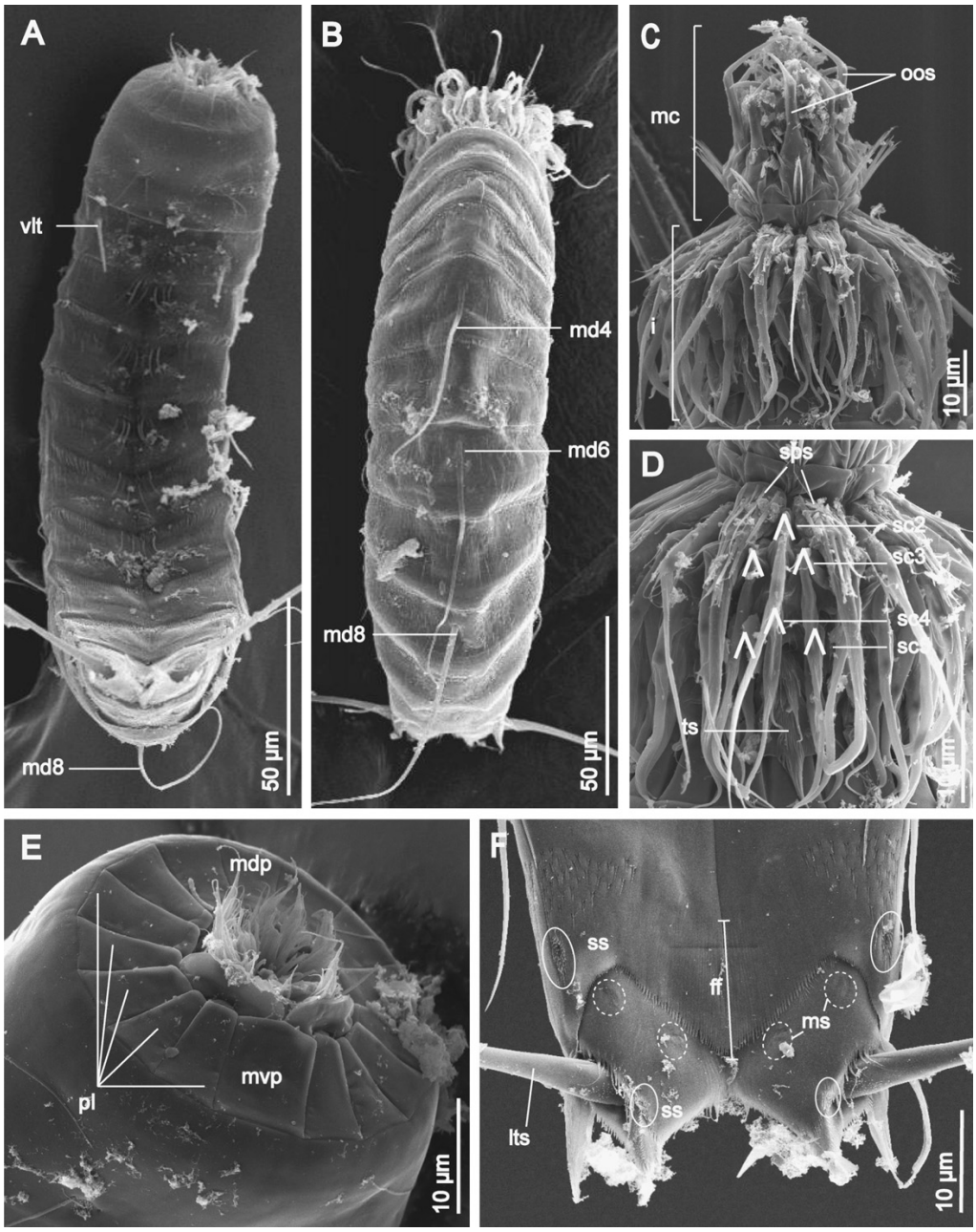

Fig. 6. *Meristoderes macracanthus* gen. et sp. nov. scanning electron micrographs. (A) Male, ventral view; (B) male, dorsal view; (C) mouth cone and introvert, showing section 1; (D) introvert, showing section 2; (E) lateroventral view of placids; (F) male, trunk segments 10–11, ventral view. Abbreviations: ff, free flap; i, introvert; lts, lateral terminal spine; mc, mouth cone; md; middorsal spine; mdp; middorsal placid; ms, muscular scar; mvp, midventral placid; oos, outer oral styles; pl, placid; sc, scalid; sps, spinoscalid; ss, sensory spot; tr, trichoscalid; vlt, ventrolateral tubule.

The introvert has seven rings of scalids and one additional ring of trichoscalids that are associated with the placids (Fig. 4). Ring 1 has 10 spinoscalids consisting of two segments. The base of each spinoscalid is equipped with two rows of long fringes, each one with four long spines (Fig. 6C and D). Ring 2 is formed by 10 laterally flattened scalids, all formed by a proximal part covered with a sheath that terminates into a fringe. Ring 3 with 20 scalids all with conspicuous sheaths around their proximal halves. These sheaths are like a gable roof with a long flexible spine in the middle. Rings 4 and 5 consist of 10 and 20 scalids, respectively. All scalids in rings 4 and 5 resemble those of ring 2. Ring 6 is formed by 6 scalids only, located with one in each odd-numbered section, and one in section 6; scalids are shorter than those in preceding rings and show shorter sheaths. The 7th ring has 10 scalids with a wide and hairy base from where several flexible elongations arise; the scalids are located as pairs in sections 1, 3, 6, and 9, and as single ones sections 5 and 7. Ring 8 has 6 trichoscalids on the respective trichoscalid plates. Two of them can be observed from the ventral side (over

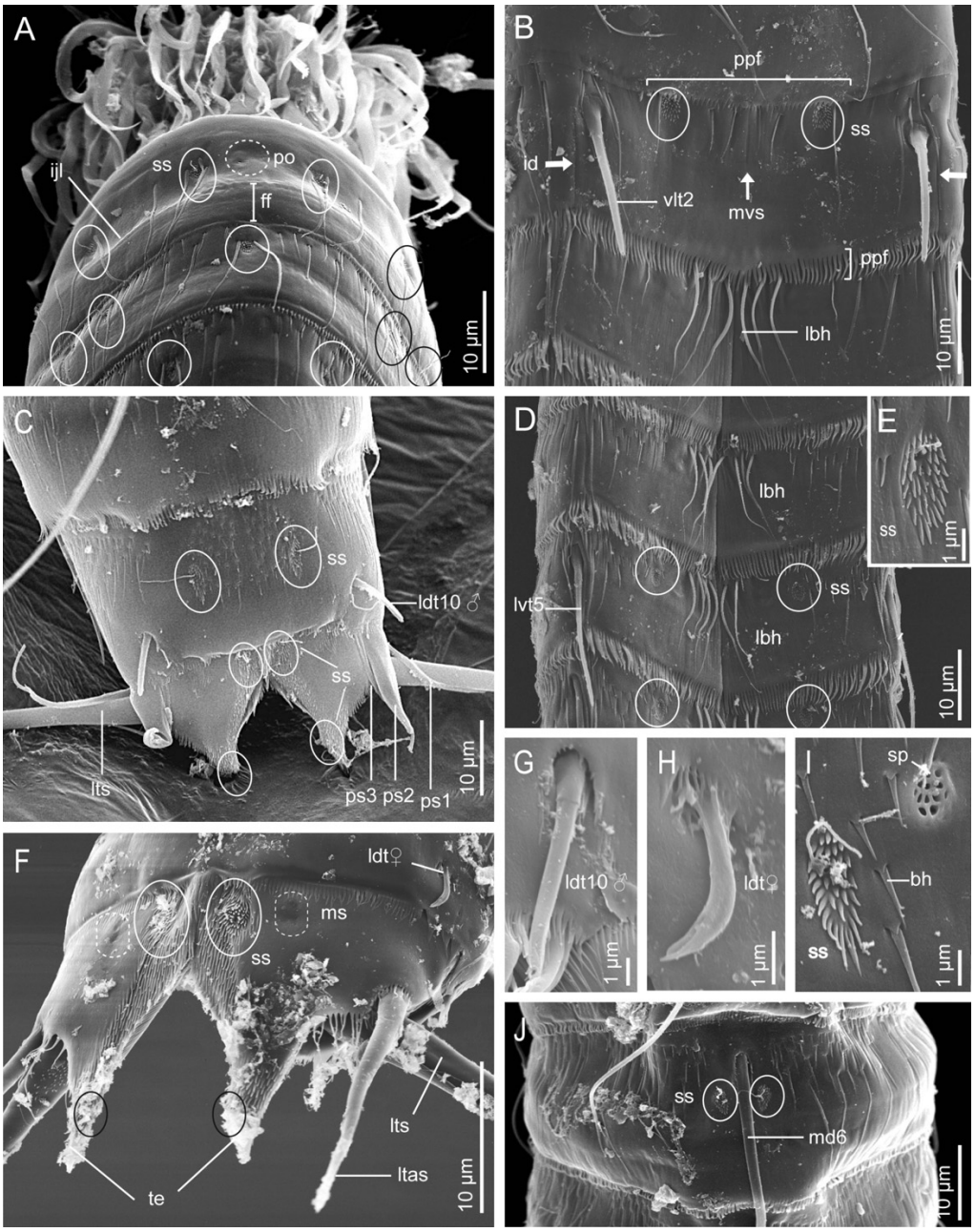

Fig. 7. *Meristoderes macracanthus* gen. et sp. nov. scanning electron micrographs. (A) Trunk segments 1–3, dorsal view; (B) trunk segments 1–3, ventral view; (C) male, trunk segments 9–11, dorsal view; (D) trunk segments 4–5, ventral view; (E) detail of a midventral sensory spot; (F) female, trunk segments 10–11, dorsal view; (G) male, detail of subdorsal tubule on segment 10; (H) female, detail of subdorsal tubule on segment 10; (I) trunk segment 9, detail of the sieve plate and a midlateral sensory spot; (J) trunk segment 6, dorsal view. Abbreviations: bh, bracteate hair; ijl, intersegmentary joint-line; ff, free flap; lbh, long bracteate hair; ldt, laterodorsal tubule; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; ms, muscular scar; mvs, midventral split; p1–3, penile spine; id, incomplete division; ppf, primary pectinate fringe; po, pore field; sp, sieve plate; ss, sensory spot; te, tergal extension; vlt, ventrolateral tubule. Digits after abbreviations refer to segment number.

placids 2 and 16) and 4 from the dorsal side (over placids 6, 8, 10 and 12).

The introvert can also be described as divided into ten sections defined by radii drawn through spinoscalids. Each section is delimited by two consecutive spinoscalids (Fig. 4). The midventral section is numbered as 1, followed clockwise by sections 2–10 (Fig. 6D). All sections, except the middorsal section 6, contain one oral style. Sections 1, 3, 6, 9 contain 9 scalids; sections 5 and 7 contain 8 scalids and also one trichoscalid. Apparently, the trichoscalid in these two sections prevents the appearance of the second scalid of ring 7; otherwise, all uneven sections (plus section 6) would have the same scalid numbers. Sections 2, 4, 8, 10 have 6 scalids and one trichoscalid. See Fig. 4 for a complete summary of oral styles, scalids and placids locations.

Table 2
Measurements of adult *Meristoderes macracanthus* nov. gen et sp. in μm or %, including number of measured specimens (N) and standard derivation (SD).

Character	*n*			Range			Average			SD		
	♀	♂	♂♀	♀	♂	♂♀	♀	♂	♂♀	♀	♂	♂♀
TL	15	16	31	235–313	244–311	235–313	266.9	274.1	270.6	22.8	20.1	21.5
SW	15	16	31	45–48	44–49	44–49	46.5	45.9	46.2	1.0	1.4	1.2
SW/TL	15	16	31	16–21%	15–19%	15–21%	18%	17%	17%	1%	1%	1%
MSW	15	16	31	53–57	54–58	53–58	54.9	54.5	54.7	1.1	1.2	1.2
MSW/TL	15	16	31	18–24%	18–22%	18–22%	21%	20%	20%	2%	1%	1%
S1	15	16	31	33–36	33–37	33–37	33.8	33.8	33.8	1.0	1.1	1.0
S2	15	16	31	27–33	26–32	26–33	29.8	29.3	29.5	2.2	1.6	1.9
S3	15	16	31	24–32	26–33	24–33	27.9	28.7	28.3	2.5	2.0	2.3
S4	15	16	31	25–32	26–32	25–32	28.6	29.0	28.8	2.3	2.1	2.2
S5	15	16	31	28–35	26–37	26–37	30.9	31.2	31.1	2.1	2.9	2.5
S6	15	16	31	29–38	29–39	29–39	32.0	32.3	32.1	2.9	2.7	2.8
S7	15	16	31	31–42	31–43	31–43	34.0	34.8	34.4	2.9	3.4	3.2
S8	15	16	31	35–43	31–46	31–46	37.8	39.5	38.6	2.6	3.6	3.1
S9	15	16	31	35–46	35–47	35–47	39.0	39.2	39.1	3.3	3.6	3.5
S10	15	16	31	42–47	36–44	36–47	43.7	39.8	41.7	1.4	2.1	1.8
S11	15	16	31	33–39	32–41	32–41	35.3	34.2	34.7	1.6	2.8	2.2
MDS 4	15	11	26	69–100	75–93	69–100	83.2	85.6	84.2	8.0	5.6	6.8
MDS 6	10	12	22	87–120	90–113	87–120	98.9	99.5	99.2	9.2	6.8	8.0
MDS 8	8	11	19	95–136	103–130	90–136	120.5	117.1	118.5	12.6	8.2	10.4
VLT 2	10	12	22	18–23	20–29	18–29	20.5	21.0	20.8	1.7	2.8	2.3
LVT 5	12	12	24	22–29	22–28	22–29	26.0	24.9	25.4	1.9	1.9	1.9
LVS 6	12	14	26	38–49	38–49	38–49	43.6	44.4	44.0	3.1	3.5	3.3
LVS 7	12	16	28	44–55	42–57	42–57	49.1	48.8	48.9	4.0	3.8	3.9
LVS 8	11	16	27	47–61	47–63	47–63	54.6	52.5	53.4	4.0	4.5	4.2
LAT 8	10	12	22	21–28	25–29	21–29	25.1	25.7	25.4	2.3	1.3	1.8
LVS 9	15	16	31	33–42	32–39	32–42	37.8	34.5	36.1	3.1	1.9	2.5
P1(♂)	IA	10	IA	IA	18–22	IA	IA	19.3	IA	IA	1.3	IA
P2(♂)	IA	2	IA	IA	16–21	IA	IA	18.6	IA	IA	3.5	IA
P3(♂)	IA	9	IA	IA	24–28	IA	IA	25.8	IA	IA	1.4	IA
LTS	10	8	18	220–254	198–224	198–254	230.8	209.5	221.4	9.9	8.1	9.0
LTAS(♀)	14	0	15	32–39	IA	32–39	34.9	IA	34.9	2.0	IA	2.0
LTS/TL	10	8	18	80–100%	71–88%	71–100%	88%	52%	84%	6%	11%	8%
LTAS/TL	14	0	14	12–15%	IA	12–15%	13%	IA	13%	1%	IA	1%

Abbreviations: IA: inapplicable; LAT: lateral accessory tubule; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; LVS: lateroventral spine; MDS: middorsal spine; MSW: maximum standard width; P: penile spine; SW: standard width; S1–S11: segment lengths of trunk segments 1–11; TL: trunk length; VLT: ventrolateral tubule; (♀): female condition of sexually dimorphic character; (♂) male condition of sexually dimorphic character.

The neck consists of 16 placids numbered clockwise from the midventral 1. All placids measure 4 μm in length. Placids 2–16 are trapezoid, 7 μm wide at the base, while placid 1 (midventral) is perfectly rectangular and wider, measuring 10 μm (Figs. 5G and 6E). All placids articulate with the first trunk segment. Trichoscalid plates with trichoscalids appear on dorsal placids 6, 8, 10, 12 and on ventral placids 2 and 16. Dorsal trichoscalid plates are rounded and small (6 μm wide) while ventral plates are larger and triangular, with enlarged bases (11 μm wide) (Figs. 3A and B, and 5B and G).

Trunk. Divided into 11 segments (Figs. 3A and B, 5A and B, and 6A and B). Segment 1 consists of one closed cuticular ring, and segment 2 of one closed cuticular ring with incomplete tergosternal divisions and a midventral fold (Figs. 3A, 5A, B and F, and 7B); segments 3–11 are composed of one tergal and two sternal plates.

The ventral part of the posterior margin of segment 1 is smooth, except for a short primary pectinate fringe in the ventromedial and paraventral areas; fringe with short triangular fringe tips (Fig. 7B). A primary pectinate fringe with short triangular fringe tips is present dorsally (Fig. 7A). Primary pectinate fringes of segments 2–9 appear similar to each other, with fringe tips in ventromedial and paraventral positions slightly shorter. Fringe tips in segments 2–5 tapering gradually, with those of segments 6–9 being more palisade-like (retaining same width until the tip). Primary fringe of segment 10 is long near midventral junction and short near tergosternal junction (Fig. 6F). Posterior fringe of segment 11 longer and more flexible,

Table 3
Summary of nature and location of spines and sensory spots arranged by series in *Meristoderes macracanthus* nov. gen et sp.

Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	PO		SS		SS				PO	SS
2	PO,SS			SS	SS				TUB	PO,SS
3	PO		SS			SS				PO
4	AC	PO								PO
5	PO		SS		SS			TUB		PO,SS
6	AC	PO,SS			SS			AC		PO,SS
7	PO		SS		SS			AC		PO,SS
8	AC	PO,SS					TUB	AC		PO
9	SPO	PO,SS	SS		SS	SP		AC	SS	PO
10	PO,PO		SS	TUB					SS	PO
11	PO	SS	SS				LTAS(♀)	LTS	SS	

Abbreviations: LA: lateral accessory; LD: laterodorsal; LV: lateroventral; MD: middorsal; ML: midlateral; PD: paradorsal; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial. AC: acicular spine; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; PO: pore field; SP: sieve plate; SPO: single pore opening; SS: sensory spot; TUB: tubule. (♀) female condition of sexually dimorphic character.

especially in the midventral split between the two sternal plates, and around the lateral terminal spines (Fig. 6F).

Secondary pectinate fringe absent on segment 1. Secondary fringes of segments 2–10 similar, consisting of one single band on each segment: entire fringe, except for a minute middorsal split, with teeth that alternate in length. Secondary fringe of segment 11 present, but predominantly hidden under primary pectinate fringe of previous segment.

Glandular pore fields are all paired, and present on the anterior part of the segments. Ventral pore fields are slightly elongated and with numerous pores (15+). They are present in a ventrolateral position on segment 1, and ventromedial position on segments 2–10; no pore fields are present on segment 11. Those of segments 2–10 always occur in a ventromedial position anterior and mesial to the perforation site cluster that extends from the tergosternal junction towards the midventral junction (Figs. 3A, and 5B, D and E). Dorsal pore fields are round, have numerous pores (more than 10) and are always located anterior to the perforation sites (Figs. 3B and 5A and C). They are unpaired middorsally on segments 1–3, 5, 7 and 11, and paired in paradorsal position on segments 4, 6, 8 and 9. Segment 10 has two unpaired middorsal pore fields above each other (Figs. 3B and 5C).

Segment 1 consists of one closed, uniformly rounded ring. The free flap is wide and delimited anteriorly by a conspicuous ij-line (Fig. 3A and B). Three pairs of round sensory spots with two pores, consisting of numerous short papillae and at least one cilium present in subdorsal, midlateral and ventromedial positions; the sensory spots appear to be flanked by long paired hairs (Figs. 3A and B, and 7A). Cuticular hairs of segment 1 originate from rounded perforation sites without bracteae and are sparsely scattered over the surface.

Segment 2 consists of a closed ring with a wide free flap which terminates into a well-developed pectinate fringe (Figs. 3A and B, 5F, and 7B). This character is constant in segments 2–9. The segment is partly divided by two incomplete tergosternal fissures (10 μm) that occur in a medial position on the segment and continue posteriorly to its posterior margin. These fissures are visible both with LM and SEM (Figs. 5F and 7B). Additionally, a partial midventral split is present in the central part of the segment, but it does not connect with either the anterior or posterior segment margin. It is also visible in both LM and SEM (Figs. 5F and 7B). One pair of thick tubules is present in a ventrolateral position; each tubule consists of a short and smooth basal part, and a longer distal part with two small wing-like lateral projections (Figs. 3A, 5F, 6A and 7B). Five sensory spots flanked by two hairs (perforation sites) are present on the dorsal side: a single middorsal one, and a laterodorsal and midlateral pair (Figs. 3B and 7A). The ventral side has one pair of round, ventromedial sensory spots, each with a single bracteate hair (Figs. 3A and 7B). All cuticular hairs on this and all following segments are bracteate, with posteriorly directed hairs emerging through a slit, and are grouped into characteristic clusters and patches. A paired paraventral cluster pattern of 4–5 perforation sites appears very distinct.

Segment 3 and the following eight segments consist of one tergal and two sternal plates. Two pairs of subdorsal and sublateral sensory spots with two associated perforation sites present (Figs. 3A and B, and 7A). Each sternal plate has a paraventral patch of perforation sites arranged into 2–4 rows, with the posteriormost row formed by three conspicuously long bracteate hairs (Figs. 3A, 5B and 7B). This character is identical on segments 3–7 (Figs. 3A, 5B and 7D). Other cuticular hairs of the segment are also bracteate and arranged in transverse lines.

Segment 4 with long, thin and flexible middorsal spine arising from a cuticular, minutely fringed notch (similar in segments 6 and 8) (Figs. 3B, 5A and 6B). No sensory spots are present. Other characters, including paraventral hair clusters, similar to previous segment.

Segment 5 with long, lateroventral tubules halfway down the segment, with the same appearance as those of segment 2 (Figs. 3A, 5B and 7D). Two pairs of sensory spots present in subdorsal and midlateral positions; the subdorsal ones with one pair of associated hairs, but only one hair for midlateral ones (Fig. 3B). Ventromedial sensory spots are slightly oval and without associated hairs (Fig. 7E). The arrangement of the cuticular hairs shows a distinctive pattern, and is distributed into two subdorsal patches and two more lateral areas. Hairs on the sternal plates grouped into V-shaped areas (Fig. 3A). Paraventral hair clusters as on segment 4.

Segment 6 with a long, thin and flexible middorsal spine as on segment 4. It is flanked paradorsally and slightly posterior, by a pair of large (up to 5 μm) oval sensory spots, with a long cilium from each of its two pores (Figs. 3B and 7J). Long acicular spines are located in a lateroventral position halfway down the segment. Midlateral and ventromedial sensory spots as on segment 5. Other characters, including cuticular hair patterns and clusters similar to those on the previous segment.

Segment 7 with long lateroventral spines halfway down the segment (Figs. 3A and 5D). Sensory spots pattern identical to segment 5, midlateral sensory spots without associated cuticular hair (Figs. 3A and B, and 5D). Other characters, including cuticular hair patterns and clusters are similar to those on the previous segment.

Segment 8 with long, thin and flexible middorsal spine, flanked by a pair of paradorsal sensory spots similar to those on segment 6 (Figs. 3B and 6B). No other sensory spots are present. Lateroventral spines halfway down the segment, and lateral accessory tubules with the same structure of those in segments 2 and 5 present (Fig. 5B and D). Paraventral cluster of cuticular hairs not arranged into lines and without longer conspicuous bracteated hairs from the posterior row (Figs. 3A and 5B). Other characters similar to those on the previous segment.

Segment 9 with shorter lateroventral spines halfway down the segment (Fig. 5B and E). Four pairs of sensory spots are present: Paradorsal sensory spots are similar to those on segments 6 and 8, with 2 cilia and a single pore opening in between. In addition, subdorsal, midlateral and ventrolateral sensory spots are oval and without associated perforation sites (Figs. 3A and B, and 5B, C and E). Paired sieve plates are present in sublateral position; sieve plates are rounded with approximately 12 openings (Fig. 7I). Cuticular hair patterns similar to those on the previous segment.

Segment 10 without spines. Males and females with a pair of laterodorsal tubules, shorter and soft in females with fringed ends and without the typical structure showed in males (Figs. 3B and E, and 7C and F–H). The posterior margin of the segment has a free flap with a midventral extension covering almost beyond the midventral margin of segment (Figs. 3A and 6F). The primary pectinate fringe is very short ventrolaterally, but increases in length towards the midventral position. One pair of subdorsal and ventrolateral sensory spots present, similar to those on segment 9 (Figs. 5C and 7C). Sternal plates lack a paraventral perforation site cluster and the ventrolateral/ventromedial clusters have a slightly different shape, being broadest at the tergosternal junction, and narrowing towards its ventromedial side (Fig. 3A and B). Tergal plate has a paired paradorsal oval perforation site cluster (sometimes fused as a single middorsal cluster) and paired lateral clusters that continue to the tergosternal junction on each side (Figs. 3A and B, 5E, and 6F). Paraventral muscle scars are distinctly large and oval in LM (Fig. 5E).

Segment 11 with one pair of long and flexible lateral terminal spines (Fig. 3C). Tergal extensions are pointed and with a distinct notch on the medial side, about halfway on the part that extends over the sternal plates (Figs. 3A and B, 6F, and 7C and F). Cuticular hair-like extensions and fringes cover the borders of the tergal

extensions. The posterior margin of the sternal plates is gently rounded and has a primary pectinate fringe, but no other conspicuous structures. Lateral edge of sternal plates with paired small and round ventrolateral sensory spots (Fig. 6F). One pair of subdorsal sensory spots is present on the notch of the tergal extensions. Larger paradorsal sensory spots are present adjacent to the middorsal fringed area of the tergal plates (Figs. 3B and D, and 7C and F). Two unidentified structures are present in ventromedial and ventrolateral positions. These are most likely scars from muscle attachments (Fig. 6F). Females with paired long lateral terminal accessory spines (Figs. 3A and B, and 7F). Males with three pairs of penile spines, P1 and P3 longer than P2 which are shorter and often thicker (Figs. 3D and E, and 7C).

3.4. *Meristoderes galatheae* sp. nov. (Figs. 8–10 and Tables 4 and 5)

3.4.1. Diagnosis

Middorsal spine on segment 4, ventrolateral tubules on segment 2, lateroventral tubules on segment 5; lateroventral spines on segments 6–9; lateral accessory tubules on segment 8; subdorsal tubules on segment 10.

3.4.2. Etymology

The species is named after the Danish scientific expedition "Galathea 3", during which it was collected.

3.4.3. Type locality

The diving locality named "Hot Spot" near Ghizo Island, Solomon Islands: GAL3-070106-HS-03: position: 08°01.781′S 156°48.850′E at 28 m depth (Fig. 1B–D). Substrate was coral sand from beneath a coral overhang.

3.4.4. Type material

Holotype: adult male (ZMUC KIN-393), mounted in Fluoromount G® (Fig. 8A and B). Allotype: adult female (ZMUC KIN-394) mounted in Fluoromount G®. Paratypes: one adult male (ZMUC KIN-395) mounted in Fluoromount G®, and one adult male (ZMUC KIN-396) mounted for SEM. All type material was obtained from a single sample taken on January 6th, 2007 by SCUBA diving at the type locality. Temperature at 28 m depth was 29 °C and salinity 35‰. Type material is deposited at the Zoological Museum, Natural History Museum of Denmark, University of Copenhagen.

3.4.5. Description

The adult specimen consists of a head, a neck and eleven trunk segments (Figs. 8A–D and 9E). Measurements and dimensions are given in Table 4. A summary of spine, tubule and sensory spot locations is given in Table 5.

Table 4
Measurements of adult *Meristoderes galatheae* sp. nov.

Specimen	HOLO male KIN-393	ALLO female KIN-394	PARA male KIN-395
TL	276	261	252
SW-10	39	44	43
SW/TL	17%	17%	14%
MSW-6	50	50	56
MSW/TL	18%	19%	22%
S1	30	30	22
S2	26	24	25
S3	24	21	22
S4	24	22	24
S5	25	24	25
S6	27	26	26
S7	28	28	31
S8	32	31	35
S9	34	31	26
S10	37	29	26
S11	30	32	32
MDS 4	20	20	–
VLT 2	16	17	13
LVT 5	15	14	15
LAT 8	16	–	16
SDT 10	11	10	11
LVS 6	14	14	16
LVS 7	14	17	17
LVS 8	15	17	17
LVS 9	14	16	17
P1 (♂)	19	IA	15
P2 (♂)	15	IA	10
P3 (♂)	29	IA	29
LTS	167	188	181
LTAS (♀)	IA	45	IA
LTS/TL	61%	72%	72%
LTAS/TL	IA	17%	IA
LTAS/LTS	IA	24%	IA

Measurements in μm or % of trunk length (TL) when stated. *Abbreviations*: IA: inapplicable; LAT: lateral accessory tubule; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; LVS: lateroventral spine; LVT = lateroventral tubule; MDS: middorsal spine; MSW = maximum sternal width; PS, penile spine; S1–11: segment lengths of trunk segments 1–11; SDT, subdorsal tubule; SW, standard width; VLT = ventrolateral spine; TL: trunk length; (–) could not be measured, but present; (♀) female condition of sexually dimorphic character; (♂) male condition of sexually dimorphic character.

3.4.6. Head and neck

Information was insufficient to describe the mouth cone and introvert morphology based on the available specimens.

The neck consists of sixteen placids. Most placids, except the midventral one, are equally sized, measuring 9 μm in length and 6 μm in width. The midventral placid is broader, measuring 9 μm in width. All placids articulate with trunk segment 1. Trichoscalid

Table 5
Summary of nature and location of spines and sensory spots arranged by series in *Meristoderes galatheae* sp. nov.

Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	PO		SS	SS					PO	SS
2	PO,SS		GO	SS				GO	TUB	PO,SS
3	PO		SS			SS				PO
4	AC	PO								PO
5	PO		SS					TUB		PO,SS
6	SPO	PO,SS			SS			AC		PO,SS
7	PO		SS		SS			AC		PO,SS
8	SPO	PO,SS					TUB	AC		PO
9	SPO	PO,SS	SS		SS		SP	AC	SS	PO
10	PO,PO		SS,TUB						SS	PO
11	PO						LTAS (♀)	LTS	SS	

Abbreviations: LA: lateral accessory; LD: laterodorsal; LV: lateroventral; MD: middorsal; ML. midlateral; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial; AC: acicular spine; GO: Gland outlet type 2; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; PO: pore field; SP: sieve plate; SPO: single pore opening; SS: sensory spot; TUB: tubule; (♀) female condition of sexually dimorphic character.

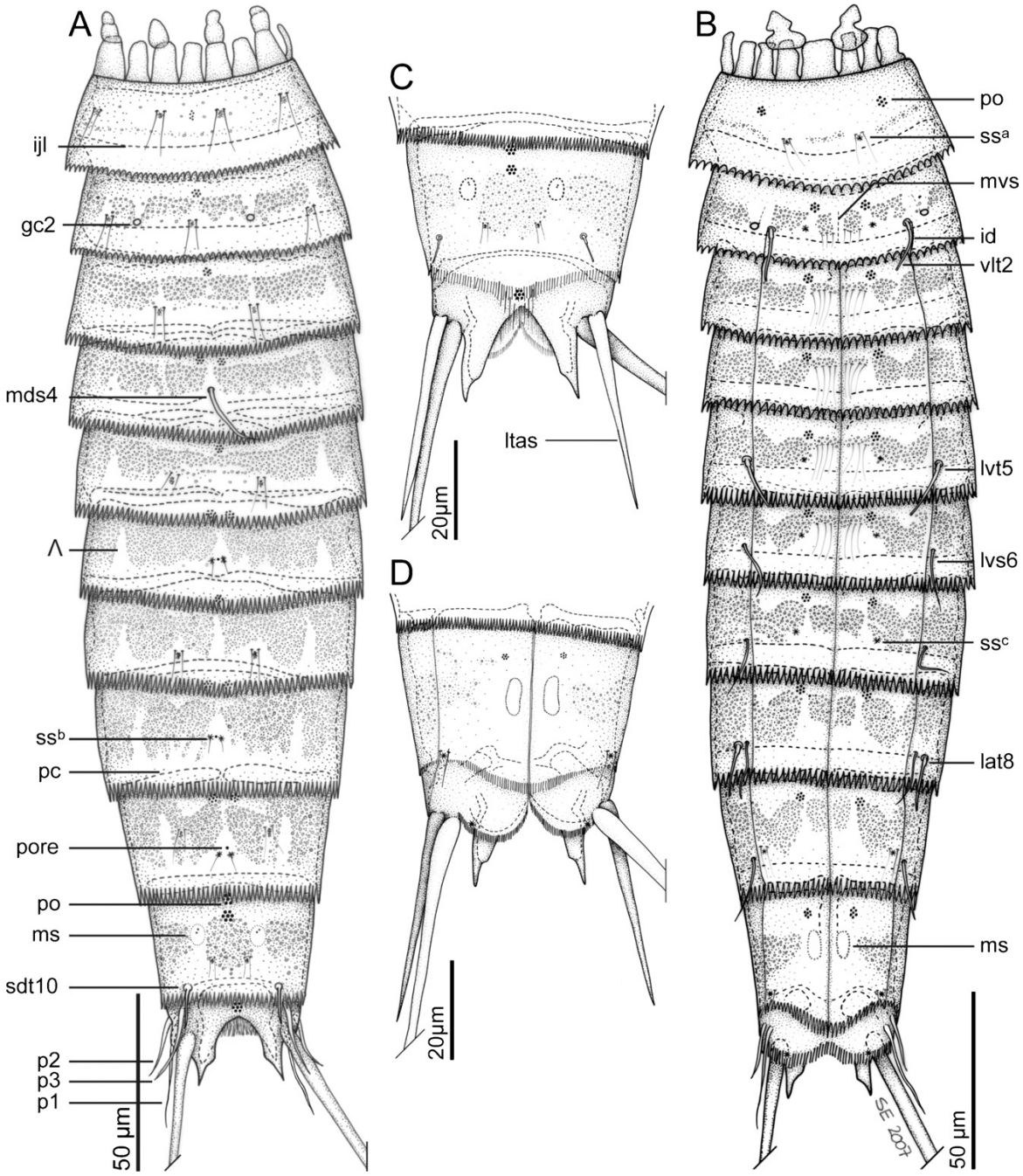

Fig. 8. *Meristoderes galatheae* sp. nov. line art illustrations. (A) Male, dorsal view; (B) male, ventral view; (C) female, trunk segments 10–11, dorsal view; (D) female, trunk segments 10–11, ventral view. Abbreviations: gc2, gland cell outlet type 2; id, incomplete division; ijl, intersegmentary joint line; lat, lateral accessory tubule; ltas, lateral terminal accessory spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; ms, muscle scar; mvs, midventral split; p1–3, penile spines; pc, pachycyclus; po, pore field; sdt, subdorsal tubule; ss, sensory spot, superscript refers to the sensory spot subtype (a, b, c); vlt, ventrolateral tubule; Λ, inverted V-shaped area without perforation sites.

plates with trichoscalids on dorsal placids number 6, 8, 10, 12 and on ventral placids 2 and 16 (Fig. 8A–B).

3.4.7. Trunk

Segment 1 consists of one closed cuticular ring, segment 2 consists of one closed cuticular ring with incomplete tergosternal divisions and a midventral fold (Figs. 8A and B, 9A, and 10F); segments 3–11 are composed of one tergal and two sternal plates.

Primary pectinate fringe of segment 1 smooth ventrolaterally, with short triangular fringe tips in the dorsal, ventromedial and paraventral areas (Fig. 10F). Primary fringes of segments 2–9 appears similar to each other; however, fringe tips of segments 2–5 tapers gradually, and those of segments 6–9 are more palisade-like

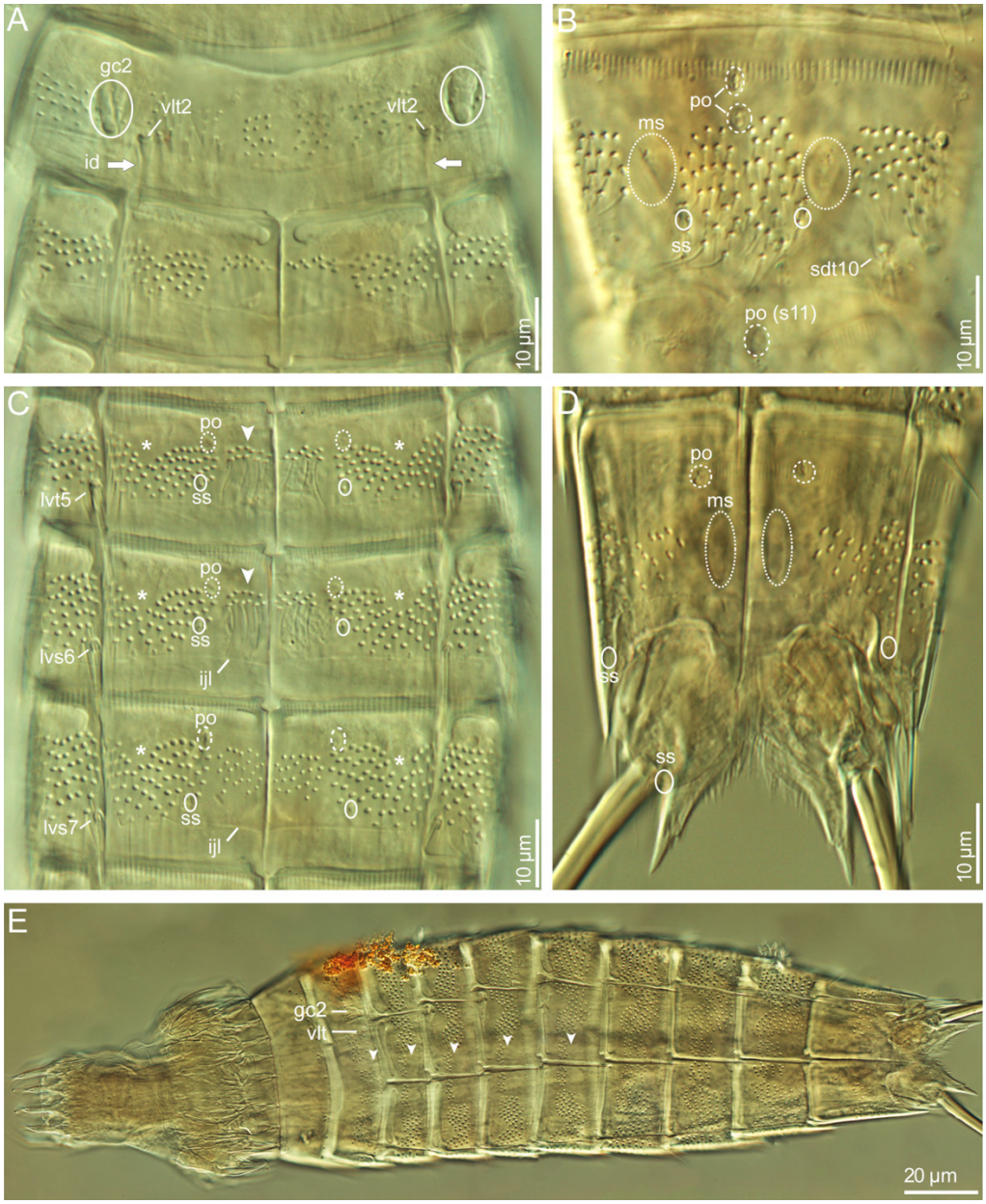

Fig. 9. *Meristoderes galatheae* sp. nov. light microscope photos including (B, D and E) male holotype (ZMUC KIN-393) and (A and C) female allotype (ZMUC KIN-394). (A) Ventral view segments 2–3. (B) Dorsal segment 10. (C) Ventral segments 5–7. Note paraventral perforation sites clusters (indicated by arrowheads), and V-shaped incision in hair pattern (indicated by asterisks *). (D) Ventral segment 10–11. (E) Ventral view. Composite of 7 images. Abbreviations: gc2, glandular cell outlet type 2; id, incomplete division; ijl, intersegmentary joint line; lvs, lateroventral spine; ms, muscular scar; po, pore field; s11, segment 11; sdt, subdorsal tubule; ss, sensory spot; vlt, ventrolateral tubule. White circles indicate pore fields (dotted line) and sensory spots (solid line).

(retaining same width until close to the tip). Fringe tips in ventromedial and paraventral positions of segments 2–9 are slightly shorter than the other fringe tips on the segment. Primary fringe of segment 10 is long near midventral junction and short near tergosternal junction (Fig. 10E). Posterior fringe of segment 11 longer and more flexible (Fig. 10E).

Secondary pectinate fringes are usually present close to the anterior edge of every segment except the first one, but hidden under primary pectinate fringe of previous segments. Secondary fringes of segments 2–10 similar, consisting of one single band on each segment: entire fringe, except for a minute middorsal split, with evenly distributed teeth of different lengths (Fig. 10D).

Pore fields are located in the anterior third of the segments and show numerous pores (15+). Ventral, slightly elongated, pore fields are present in a ventrolateral position on segment 1, ventromedial position on segments 2–10, and none on segment 11. Those of segments 2–10 always occur in a ventromedial position anterior and mesial to the perforation site cluster that extends from the tergosternal junction towards the midventral junction (Figs. 8A and B, 9C and D, and 10G). Dorsal pore fields are round

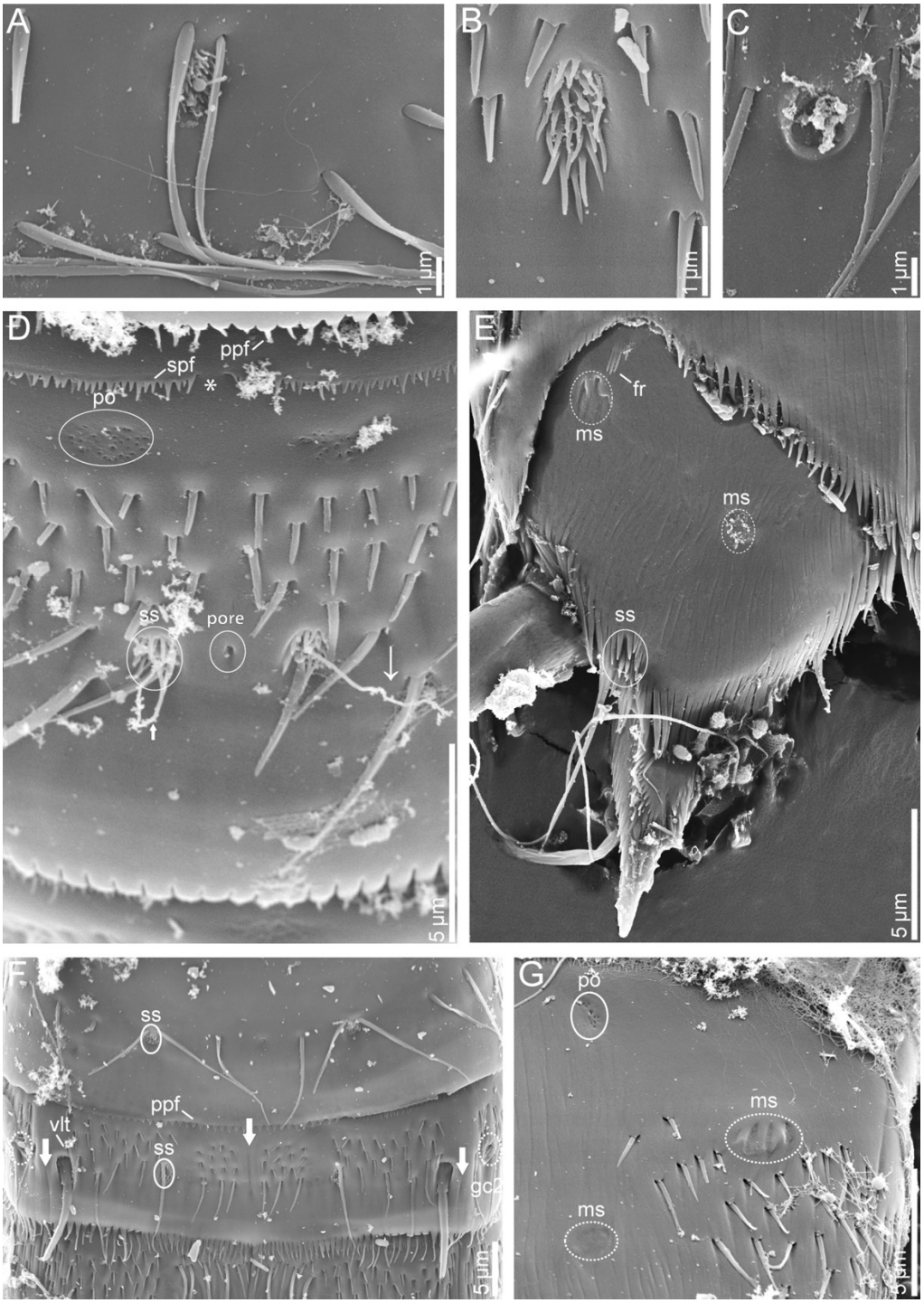

Fig. 10. *Meristoderes galatheae* sp. nov. scanning electron micrographs of male. (A) Sensory spot with two anterior hairs in a subdorsal position on segment 1. (B) Sensory spot in a ventromedial position on segment 6. (C) Glandular cell outlet (type 2) in a ventrolateral position on segment 2. (D) Segment 6, dorsal view. Note middorsal split of secondary pectinate fringe (asterisk), single middorsal pore (pore) and sensory spots, with long cilium from each sensory spot (arrow) and microspine. (E) Ventral segment 11 with sensory spot at the posterior edge of the sternal plate. (F) Ventral segment 1–2 showing partial tergosternal splits (arrow) and incomplete midventral articulation (arrow). Note triangular primary pectinate fringe on segment 1 and paraventral hair cluster of segment 2. (G) Sternal plate of segment 10 with distinct muscle scars. Abbreviations: fr, fringe; gc2, glandular cell outlet type 2; ms, muscle scar; po, pore field; ppf, primary pectinate fringe; spf, secondary pectinate fringe; ss, sensory spot; vlt, ventrolateral tubule.

and always located anterior to the perforation sites (Fig. 10D). They are unpaired middorsally on segments 1–3, 5, and 7 and paired in a paradorsal position on segments 4, 6, 8 and 9. Segment 10 has two unpaired middorsal pore fields above each other, while segment 11 has at least one middorsal pore field, possibly two (Fig. 9B).

Segment 1 consists of one complete cuticular ring. In cross-section the segment is uniformly rounded. Paired sensory spots are present in subdorsal and laterodorsal positions, at the same level as the middorsal pore field. These sensory spots are anteriorly flanked by two long hairs (Fig. 10A). Similar sensory spots are located in a ventromedial position, one third from the posterior margin, in line with the transverse band of perforation sites (Fig. 10F). Cuticular hairs from perforation sites in this segment are non-bracteate long acicular hairs emerging through a circular pore (Fig. 10A and F). They are mainly arranged in a single row around the whole segment (Figs. 8A and B, and 10F) and the dorsal side has an additional irregular row of hairs.

Segment 2 consists of one complete cuticular ring which is more flattened ventrally than segment 1. The segment is partly divided by two incomplete tergosternal fissures (10 μm), which begin externally in a medial position and extend posteriorly to the posterior margin of the segment (Figs. 8B, 9A and 10F). These fissures are distinct in SEM, but could not be easily observed with LM (Fig. 9A). Additionally, a partial midventral split visible with both LM and SEM is present in the central part of the segment, but does not connect with either the anterior or posterior margin of the segment (Figs. 9A and 10F). Paired tubules are present in ventrolateral positions, in line with the anterior ends of the tergosternal fissures, the posteriormost perforation sites and the sensory spots (Figs. 8B, 9A and 10F). Distinct large single openings of glandular cell outlets are present in subdorsal and lateroventral positions (Figs. 8A and B, 9A, and 10C and F). Sensory spots with two anterior associated hairs are present in middorsal and laterodorsal positions, while a pair of sensory spots with only a single anterior associated hair is present in ventromedial positions (Figs. 8A and B, and 10F). Cuticular hairs from perforation sites on segment 2 (and all posterior segments) are bracteate, posteriorly directed hairs emerging through a slit (Figs. 10B and F). Segment 2 cuticular hairs are arranged into clusters around the whole segment, with the posteriormost hairs two to ten times longer than those above (Fig. 10F). A pair of paraventral clusters form a square of 3–4 rows, with hairs in the posteriormost row much longer (Figs. 9E and 10F).

Segment 3 and the following nine segments consist of one tergal and two sternal plates. Subdorsal and sublateral sensory spots with two anterior hairs present on segment 3. Segments 3–9 have fairly uniform perforation site patterns. The sternal plates of segments 3–9 all have two perforation site clusters, one paraventral and one ventromedial/ventrolateral. The ventromedial/ventrolateral cluster has a hairless V-shaped incision from the anterior edge, denoting the borderline between the ventrolateral and ventromedial positions (Figs. 8B and 9C). The paraventral clusters on segments 3–6 consists of 2–3 rows of hairs, of which the posteriormost carry long, conspicuous acicular hairs with a length up to 15 μm (Figs. 8B and 9C).

Segment 4 with middorsal flexible spine halfway between anterior and posterior segment margins (Fig. 8A). Sensory spots absent. Cuticular hairs from perforation sites similar to segment 3.

Segment 5 with lateroventral tubules just anterior to ij-line (Figs. 8B and 9C). Ventromedial sensory spots without associated long hairs from perforation sites, posterior to the gland pore field and close to the ij-line (Figs. 8B and 9C). Subdorsal sensory spots with two anterior cuticular hairs. Cuticular hairs from perforation sites similar to segment 3.

Segment 6 with lateroventral spines just above ij-line (Figs. 8B and 9C). Midlateral sensory spots with two anterior cuticular hairs and ventromedial sensory spots without distinctly associated perforation sites (Fig. 10B). Paradorsal sensory spots with distinct long cilium from upper pore and a cuticular hair from a perforation site within or directly below the sensory spot (Fig. 10D). A single middorsal pore opening is present between the paradorsal sensory spots (Fig. 10D). Cuticular hairs from perforation sites similar to segment 3.

Segment 7 with lateroventral spines just above ij-line (Figs. 8B and 9C). Ventromedial, subdorsal and midlateral sensory spots present; two anterior cuticular hairs associated with the subdorsal and midlateral ones. Cuticular hairs from perforation sites similar to segment 3 but the paraventral clusters of segment 7 (and 8 and 9) has about 5–8 rows of very short hairs (<1 μm) without the long conspicuous hairs (Figs. 8B and 9C).

Segment 8 with lateroventral spines and lateral accessory tubules, both just above ij-line (Fig. 8B). Paradorsal sensory spots similar to those on segment 6, also with single pore opening in between. Cuticular hairs from perforation sites similar to segment 7.

Segment 9 with lateroventral spines just above ij-line (Fig. 8B). Paradorsal sensory spots similar to those on segment 6, also with single pore opening in between. Subdorsal, midlateral and ventrolateral sensory spots with 2 anterior cuticular hairs. Sieve plates paired in lateral accessory position, slightly more anterior than the lateroventral spine. The plate is minute (1–2 μm when observed in SEM) and with approximately 10 pore openings. Cuticular hairs from perforation sites similar to segment 7.

Segment 10 with subdorsal tubules close to posterior margin (Figs. 8A and 9B). Ventrolateral sensory spots with two anterior cuticular hairs close to posterior margin. Subdorsal sensory spots with two anterior cuticular hairs, at the lateral edges of the paradorsal perforation site clusters (Fig. 9B). Paraventral muscle attachment sites are large and oval in LM and located halfway down the segment (Figs. 8B and 9D). In SEM they appear as rounded, oval depressions (Fig. 10G). Segment 10 sternal plates lack a paraventral hair cluster and the ventrolateral/ventromedial cluster has a slightly different shape, being tallest at the tergosternal junction, and narrowing towards its ventromedial side (Figs. 8B and 9D). Segment 10 tergal plate has paired paradorsal oval perforation site clusters (appears together as a single middorsal cluster) and paired lateral clusters that continue to the tergosternal junction on each side (Fig. 9B).

Segment 11 with lateral terminal spines. Tergal extensions pointed and with distinct notch on medial side, about halfway on the part that extends over the sternal plates (Figs. 8A, C and D, and 9D). Posterior margin of sternal plates gently rounded with no conspicuous structures. A detached fringe of 4–5 fringe tips is present in the zone between the ventromedial and ventrolateral positions, above a muscle scar (Fig. 10E). Paired ventrolateral sensory spots without associated perforation sites or hairs are protruding slightly from the posterior margin of the sternal plates, mesially adjacent to the lateral terminal spines position (Fig. 10E). Two unknown structures are present in ventromedial and ventrolateral positions and only distinct in SEM. These are most likely scars from muscle attachments (Fig. 10E). Cuticular hairs from perforation sites are absent on segment 11. Females with lateral terminal accessory spines (Fig. 8C and D). Males with three pairs of penile spines, P1 and P3 longer than P2 which are shorter and many times thicker (Fig. 8A).

3.5. Notes on diagnostic features

The new genus *Meristoderes* is clearly distinguished from other echinoderid genera by its incomplete paired tergosternal divisions and a midventral fold on segment 2.

M. macracanthus gen. et sp. nov. shares many characters with several species of *Echinoderes*: its spine formula is identical to *Echinoderes riedli* Higgins, 1978, *Echinoderes abbreviatus* Higgins, 1983, *E. kristenseni* and *Echinoderes higginsi* Huys and Coomans, 1989 (see Higgins, 1978, 1983; Huys and Coomans, 1989). However, they all have shorter middorsal spines, and even lateroventral spines and tubules are shorter than those of *M. macracanthus*. gen. et sp. nov. This is most conspicuous when comparing the range of middorsal spine lengths (shortest middorsal spine on segment 4 to the longest middorsal spine on segment 8) of *M. macracanthus* gen. et sp. nov. (69–136 μm) with *Echinoderes riedli* (15–18 μm) and *E. abbreviatus* (13–38 μm), which also both have much shorter laterotermINAL spines and very different trunk dimensions and proportions. *Echinoderes higginsi* also has shorter middorsal spine lengths (27–82 μm), shorter and more stout conspicuous tergal extensions. Within *Echinoderes*, the most similar species to *M. macracanthus* gen. et sp. nov., from its overall appearance could be *E. kristenseni* because a more similar length of middorsal spines (60–92 μm) and other spines. A comparative study with specimens of *E. kristenseni* from Denia (East coast of Spain) (M. Herranz, unpublished observations) allowed to conclude that, apart from diagnostic characters of the new genus, this species can be discriminated from *M. macracanthus* gen. et sp. nov. by the pattern and typology of sensory spots, presence of gland openings on segment 2 and trunk dimensions.

M. galatheae sp. nov is distinguished from *M. macracanthus*. gen. et sp. nov. by having only having a short, single middorsal spine on segment 4 opposed to middorsal spines on segments 4, 6 and 8. Patterns of all other spines and tubules on segments 2–9 are identical, but those of *M. macracanthus* gen. et sp. nov. are much longer (Figs. 3A and B, and 8A and B and Tables 2 and 4). The type 2 glandular cell outlets in subdorsal and lateroventral positions on segment 2 are not present in *M. macracanthus* gen. et sp. nov. and the conspicuous long hairs from the paraventral perforation sites are present on segments 2–6 in *M. galatheae* sp. nov but on segments 2–7 in *M. macracanthus* gen. et sp. nov (Figs. 3A and 8B). Sensory spot patterns are very similar, but *M. galatheae* sp. nov does not have any midlateral sensory spots on segments 2 and 5, or the subdorsal ones on segment 11, and the paradorsal sensory spots of *M. macracanthus* gen. et sp. nov are larger.

Within the family Echinoderidae, a single middorsal spine on segment 4 is shared only with five other species: *E. cantabricus*, *Echinoderes capitatus* Zelinka, 1928, *Echinoderes isabelae* Gª Ordóñez et al., 2008, *Echinoderes rex* Lundbye et al., 2011 and *Echinoderes teretis* Brown, 1985.

E. cantabricus from Cantabria, Spain, is distinguished by the absence of lateroventral spines on segments 8–9, and dorsal tubules on segment 10 in males only instead of both sexes. It has a somewhat larger trunk length (328–408 μm compared to 252–276 μm of *M. galatheae* sp. nov) and numerous additional pairs of tubules: midlateral on segment 1, subdorsal, laterodorsal and sublateral on segment 2. Reexamination of six paratypic specimens (ZMUC KIN-62–KIN-67) furthermore revealed the presence of lateral accessory tubules on segment 8 in both sexes, instead of lateroventral tubules in females and sublateral tubules in males, as stated in the original description (Pardos et al., 1998).

According to the redescription by Nebelsick (1992b) *E. capitatus* from Italy has very little in common with *M. galatheae* sp. nov. They only share the middorsal spine on segment 4, a pair of ventrolateral tubules on segment 2, a pair of lateroventral tubules on segment 5, a pair of lateral accessory tubules on segment 8, and dorsal (laterodorsal or subdorsal) tubules on segment 10 in both sexes. *E. capitatus* differs from *M. galatheae* sp. nov. by the presence of lateroventral tubules on segments 6-9 instead of spines, and in having numerous additional tubules. In addition, *E. capitatus* has no LTAS and shorter LTS (64–88 μm = 23–33% of TL) compared to the LTS of *M. galatheae* sp. nov (167–188 μm = 61–72% of TL).

E. isabelae from Cantabria, Spain (Gª Ordóñez et al., 2008), differs from *M. galatheae* sp. nov by having three additional pairs of tubules on segment 2, a pair of sublateral tubules on segment 7 and subdorsal tubules on segment 8, and by lacking dorsal tubules on segment 10. In addition *E. isabelae* has a distinct paraventral pattern of cuticular hair-like extensions on segments 3–10, and lacks LTAS in females.

E. rex from the Korea Strait (Lundbye et al., 2011), is clearly distinguished from *E. galatheae* sp. nov by its big dimensions (482–528 μm compared to 252–276 μm of *M. galatheae* sp. nov) and the presence of a pair of diminutive lateral terminal spines (19–23 μm). In addition *E. rex* shows special type 2 glandular openings on segments 2–4 and 5–8, a conspicuously large sieve plate and lacks a ventrolateral pair of tubules on segment 2.

E. teretis from Sydney, Australia (Brown, 1985), is easily distinguished from *E. galatheae* sp. nov by its lack of lateroventral spines on segments 8 and 9, ventral tubules on segment 2 and dorsal tubules on segment 10. Furthermore, the Australian species shows a characteristic hunchback-like appearance in lateral view that differentiates it from all other species of *Echinoderes*.

None of the other echinoderid genera, *Cephalorhyncha*, *Fissuroderes* or *Polacanthoderes* contains species with only a single middorsal spine, and since only a low number of species have been described (9 in total) the potential variation in spine patterns may not yet have been revealed. When disregarding middorsal spines, a similar spine/tubule pattern can be seen in four of these species. *Fissuroderes papai* Neuhaus and Blasche, 2006 which differs by its "funnel-shaped glands", lack of large type 2 glandular cell outlets in segment 2, and most likely by a lack of conspicuous paraventral perforation site clusters, although this is difficult to conclude based on its description and images. Another species, *F. thermoi* Neuhaus and Blasche, 2006 differs, e.g., in its lack of lateral accessory tubules on segment 8, the presence of funnel-shaped glands and its tergal extensions. *Cephalorhyncha asiatica* and *C. liticola* both have middorsal spines on segment 4 to 8, but otherwise a spine pattern identical to *M. galatheae* sp. nov. They differ by not having single opening gland outlets or paraventral perforation site clusters.

4. Discussion

Meristoderes gen. nov. clearly belongs to the class Cyclorhagida Zelinka, 1896 based on the first trunk segment consisting of a closed ring and the presence of a broad midventral placid. The combination of segment 1 forming a closed ring, segments 3–11 being composed of a tergal and two sternal plates, presence of only 6 trichoscalid plates, aligned middorsal spines, absence of middorsal spines on segment 9 as well as absence of a midterminal spine, suggests close relationship of *Meristoderes* gen. nov. with the echinoderid genera *Cephalorhyncha*, *Echinoderes*, *Fissuroderes*, and *Polacanthoderes*. Hence, the new genus and species can be assigned to the same family, the Echinoderidae.

Within Echinoderidae the second trunk segment is the key to discriminate the four existing genera referred to above (Fig. 11). The second segment of *Echinoderes* forms a complete ring without divisions, while it in *Cephalorhyncha* is divided into a tergal and a single sternal plate by two lateral articulations. The sternal plate in *Cephalorhyncha* has only an incomplete, midventral division (Adrianov and Malakhov, 1999; Neuhaus and Blasche, 2006; Sørensen, 2008b; Sørensen and Pardos, 2008). *Fissuroderes* and *Polacanthoderes* both have one midventral and two lateral articulations resulting in two sternal plates and one tergal plate (Neuhaus and Blasche, 2006; Sørensen, 2008a; Sørensen and Pardos, 2008). The new genus *Meristoderes* gen. nov. differs from these genera by

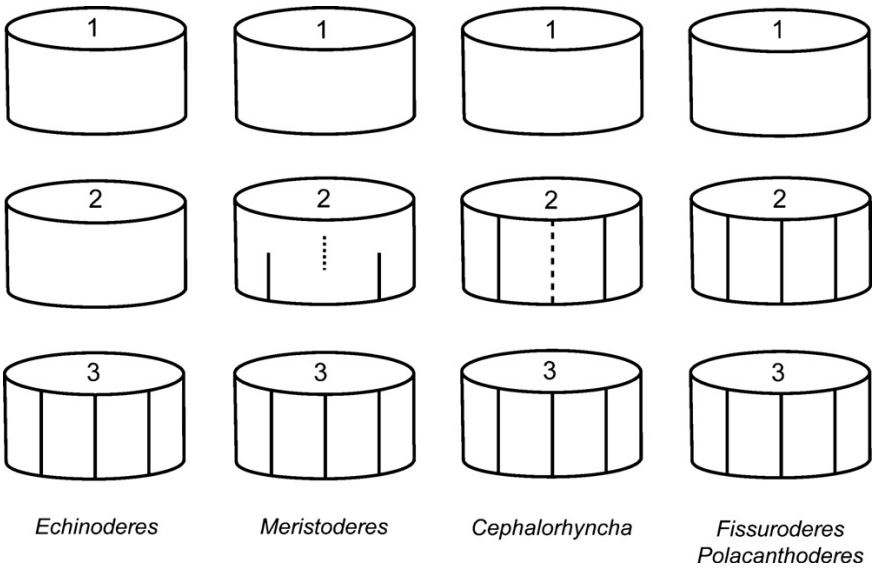

Fig. 11. Comparative diagram of the cuticular plates in the three first trunk segments of the echinoderid genera. Dotted lines represent internal cuticular divisions, that appear indistinct externally. Note the incomplete divisions in *Meristoderes* gen. nov.

its partial tergosternal division of segment 2. In addition, the midventral area of segment 2 possesses a superficial fold or furrow which does not connect with either the anterior or posterior margin of the segment.

Midventral folds on segment 2 have been reported in five Greenlandic species of *Echinoderes* (Higgins and Kristensen, 1988; Neuhaus and Blasche, 2006). The present study confirms these folds to be weak and superficial in *E. angustus*, *E. aquilonius* and *E. eximus*, using LM and SEM. Similar folds have furthermore been discovered in *Echinoderes truncatus* Higgins, 1983 (LM) and *Echinoderes intermedius* Sørensen, 2006 (J. Thormar SEM pers. obs.). However, no *Echinoderes* or any other kinorhynch genera possess partial lateral division of segment 2, and this alone justifies the erection of the new genus *Meristoderes* gen. nov.

The close relationship between *Meristoderes* gen. nov. and one or more genera within Echinoderidae is supported by several other similarities.

Acicular cuticular hairs from perforation sites are similar in the three echinoderid genera *Cephalorhyncha*, *Meristoderes* nov. gen. and most species of *Echinoderes*; they arise from round pores on segment 1 and are mostly bracteate on all subsequent segments to segment 10 (Figs. 7A and 10F). Species of *Fissuroderes* seem to share the same pattern, but this has not yet been confirmed by SEM, while the last echinoderid genus, *Polacanthoderes*, has no perforation sites or hairs (Sørensen, 2008a). Outside Echinoderidae species of *Dracoderes*, also share the same type of acicular hairs from perforation sites (Sørensen, 2008b) and these are also from round pores on segment 1 and bracteate on subsequent segments, although with two bractea instead of a single one (M. Herranz pers. obs. by SEM of undescribed *Dracoderes* spp. from Spain). This character ties *Meristoderes* nov. gen. closer to the other echinoderid genera, and also supports the suggestion by Sørensen (2008b) that *Dracoderes* may be very close to the Echinoderidae.

Both *M. macracanthus* gen. et sp. nov and *M. galatheae* sp. nov. have conspicuous paraventral clusters of acicular hairs from perforation sites (Figs. 3A, 5B, 7B and D, 8B, 9C and E, and 10F). Paraventral hair-like patterns are seen in many species of Echinoderidae but one should distinguish between those that are merely filiform cuticular extensions, and those originating from perforations sites (see Thormar and Sørensen, 2010). Paraventral clusters of bracteate hairs from perforation sites are found in most species on segment 2, and on segments 3–9 in at least 16 of the 68 species of *Echinoderes* (*E. abbreviatus* (line art illustrations in Higgins, 1983), *E. andamanensis* Higgins and Rao, 1979 (line art illustrations in Higgins and Rao, 1979), *E. bispinosus* Higgins, 1982 (J. Thormar, pers. obs. 2010), *E. brevicaudatus* Higgins, 1966 (J. Thormar, pers. obs. 2010), *E. cantabricus* (M. Herranz, pers. obs. 2010, and line art illustrations in Pardos et al., 1998), *E. coulli* Higgins, 1977 (J. Thormar, pers. obs. 2010), *E. hispanicus* (M. Herranz, pers. obs. 2010 and line art illustrations in Pardos et al., 1998), *E. horni* Higgins, 1983 (J. Thormar, pers. obs. 2010), *E. kristenseni* (M. Herranz, pers. obs. 2010), *E. maxwelli* (J. Thormar, pers. obs. 2010), *E. peterseni* (J. Thormar, pers. obs. 2010), *E. riedli* (line art illustrations in Higgins, 1978), *E. teretis* (J. Thormar, pers. obs. 2010) *E. wallaceae* Higgins, 1983 (J. Thormar, pers. obs. 2010) and *E. worthingi* (M. Herranz, pers. obs. 2010). None of the other echinoderid genera are confirmed to have paraventral perforation site patterns on segments 3–9.

The conspicuous elongated hairs from the posterior margins of the paraventral clusters, showed in both *M. macracanthus* gen. et sp. nov and *M. galatheae*, sp. nov have not been reported previously in other echinoderid descriptions but they are present at least in *E. cantabricus* and *E. hispanicus* (M. Herranz, pers. obs. 2010).

The sensory spots of the new genus *Meristoderes* are all of type 1 (*sensu* Nebelsick, 1992a). They show two pore openings at the same level surrounded by numerous papillae, which fits with the variation seen in the other echinoderid genera. The number of pores and the presence of a cilium extending from the sensory spot are difficult to confirm by SEM for all sensory spots, and such information has not been gathered consistently. Several variations within this type were observed in each of the two new species, giving a total of 4 distinct subtypes:

(a) Round or oval sensory spot with 2 associated anterior perforation sites from which two long hairs normally protrude (see *M. macracanthus* sp. nov. Fig. 7A and *M. galatheae* sp. nov. Fig. 10A).
(b) Round or oval sensory spot with 1 associated anterior perforation site (see segment 2 of *M. macracanthus* sp. nov. Fig. 7B and *M. galatheae* sp. nov. Fig. 10F).

(c) Round/oval sensory spot with no distinctly associated hairs (see *M. macracanthus* sp. nov. Fig. 7E, and *M. galatheae* sp. nov. Fig. 10B).
(d) Round/oval (1 μm) with one elongated microspine from the posterior part (see *E. galatheae* sp. nov. Fig. 10D).

The first two variations are shared with species of genus *Cephalorhyncha* and some *Echinoderes,* while the subtype 3 is widespread distributed within Echinoderidae. Subtype 4 has previously been reported once for *E. cantabricus* (Benito et al., 1994). The published descriptions of *Fissuroderes* species lack SEM photos, thus it is very difficult to assess their type of sensory spots.

The primary pectinate fringe in species of *Meristoderes* gen nov. is well developed in all segments except the first one, which shows a ventromedial area with small, triangular tips that disappear on lateroventral areas towards tergal plate (Figs. 7B and 10F). This configuration of the primary pectinated fringe is shared with the genus *Polacanthoderes*, although the latter shows bigger and more conspicuous tips on the ventromedial area. Within the genus *Echinoderes*, *E. tubilak* and *E. hispanicus* also show specializations of the primary pectinate fringe on the ventromedial area of segment one. The first one showing stronger and wider fringed tips compared to the lateroventral and dorsal area, and the second one showing weaker and thinner fringed tips compared with those in *M. macracanthus* gen et sp. nov. A similar pattern can be observed in species of the genus *Dracoderes* (M. Herranz, pers. obs. 2011). However, a comprehensive survey of kinorhynch species and taxa regarding this trait should be accomplished to evaluate its taxonomic and/or phylogenetic importance.

The two species of *Meristoderes* gen nov. show sexual dimorphism which is common for species of Echinoderidae, namely females with LTAS and males with three pairs of penile spines. In addition, secondary sexual differences are also expressed in the paired tubules located laterodorsally on segment 10 in both sexes. Those of males have the regular tubule structure which are common among species of *Echinoderes* (Figs. 3E, 7C and G, and 8A), whereas females show a special tubule with a different structure (Figs. 3B, and 7F and H). Within Echinoderidae, most species of *Echinoderes* have subdorsal tubules on segment 10, while females have none. Tubules on segment 10 in females appear in *Fissuroderes higginsi* Neuhaus and Blasche, 2006 and *Fissuroderes thermoi* Neuhaus and Blasche, 2006, but always showing the same structure as in males. In the genera *Cephalorhyncha* and *Polacanthoderes* both sexes show laterodorsal spines on segment 10. The special tubules from females of *Meristoderes* gen nov. have only been described once in a recent described echinoderid species: *E. rex* (Lundbye et al., 2011).

The tergal plate seems to have a middorsal split, being not clearly differentiated on the anterior part of the segment. This is a very difficult character to check due to the partial overlap of segment 10, the dense covering of cuticular hairs and the closeness of the sternal plates which may confuse the observer. A tergal plate split has up till now only been described in the genus *Cephalorhyncha* although this is not a very reliable character due to the difficulties presented to check it consistently, and the lack of graphic material showing it clearly. Further morphological analyses are required to check this character in all echinoderid genera and to determine its phylogenetic importance.

4.1. Phylogenetic remarks

We consider the new genus *Meristoderes* as very close to *Echinoderes* since both genera share morphological characters such as spine and hair patterns, placid number and arrangement, sensory spot structure and general distribution and even overall appearance. In fact, they could be easily confused to the unexperienced eye. On the other hand, the phylogenetic relationships among kinorhynch are yet to be resolved (Adrianov and Malakhov, 1999; Neuhaus and Higgins, 2002; Sørensen, 2008a; Sørensen and Pardos, 2008; Sørensen et al., 2009). The different types of subdivision of the cuticle into plates in the trunk segments play an important role as a key character for kinorhynch taxonomy. The condition of the second trunk segment, and in particular the development/loss of sternal plates through bilateral and midventral fissures/joints, is obviously pivotal for understanding the internal echinoderid relationships (Sørensen, 2008b).

Currently there are two hypotheses to describe the evolutionary development of the second trunk segment.

The first one assumes that the kinorhynchs stem from an ancestor with an unsegmented, tube-like body cuticle without any cuticular plates (Neuhaus, 1991, 1994; Adrianov and Malakhov, 1999; Neuhaus and Higgins, 2002; Neuhaus and Blasche, 2006). When segmentation appeared, it is simpler to assume that the kinorhynch body was formed first by segments as cuticular rings. This character is supposed to be retained, e.g., in the first two trunk segments of species of *Echinoderes*. Then, this ring-like trunk cuticle of segment 2 has in one evolutionary event separated into a single sternal plate and a single tergal plate, as in *Cephalorhyncha* and, in a subsequent evolutionary event, the single sternal plate has further divided into two sternal plates as in *Fissuroderes* (Neuhaus and Blasche, 2006).

Alternatively, recent phylogenetic analyses of Echinoderidae using morphological traits (Sørensen, 2008a) suggest that differentiation of segment 2 into one tergal and two sternal plates is a basal kinorhynch trait, and hence plesiomorphic for Echinoderidae. Comparing species of various kinorhynch groups it becomes evident that all species of the Homalorhagida and most non-echinoderid cyclorhagid species possess midventral and lateroventral/ventrolateral articulations on segment 2. In this case the most parsimonious solution would be to interpret the lack of articulated plates in the second segment as autapomorphic for *Echinoderes*, with *Cephalorhyncha* representing an intermediate stage between the complete cuticular division into two sternal and one tergal plate, as depicted in *Fissuroderes* and *Polacanthoderes*, and the closed ring found in species of *Echinoderes*.

Considering both hypotheses the new genus *Meristoderes* (with a second trunk segment with a non-complete tergosternal division and a midventral fold) most likely also represent an intermediate stage in this transformation series between the configuration in *Echinoderes* (second segment forming a closed ring) and *Cephalorhyncha* (second segment composed of one tergal and one sternal plate with a non-complete midventral division) as shown in Fig. 11. However, to complete the picture we still lack the arrow marking the correct direction of the evolutionary progress. Additionally, we should never forget that morphological traits are tied to functional activities of the living animal. In this case, the first segment as a single cuticular ring is clearly related to the cyclorhagid closing system of the trunk when the introvert is withdrawn. Undoubtedly a more rigid segment provides the needed stability for the proper infolding of placids. The cuticular division of the first trunk segment in homalorhagids fits their special closing system, approaching the dorsal and ventral edges of the first segment. To make the picture even more complicated, the so-called conchorhagids (*Semnoderes*, *Sphenoderes* and probably *Antygomonas*) show a completely different closing system, with a bilateral folding of the first segment that needs a much more soft or thin cuticle. We still do not know if this morphofunctional approach can be extended to explain the variability of the second trunk segment in cyclorhagids.

With the discovery of the new genus and two new species the variability of cuticular arrangement on segment 2 is still increasing, with a wide variety of states. Defining the polarity of this

character is always tentative without a more comprehensive phylogenetic analysis. Subsequent phylogenetic studies, including thorough systematic revisions of many genera and incorporating molecular approaches would certainly provide the solid arguments needed for a discussion on the evolutionary relationships of the phylum.

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Chapter III

On the genus *Dracoderes* Higgins & Shirayama, 1990 (Kinorhyncha: Cyclorhagida) with a redescription of its type species, *D. abei*, and a description of a new species from Spain

Estudios en el género *Dracoderes* Higgins & Shirayama, 1990 (Kinorhyncha: Cyclorhagida) incluyendo la redescripción de la especie tipo, *D. abei*, y la descripción de una nueva especie en España

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En este trabajo se redescribe la especie tipo del género *Dracoderes*, *D. abei*, a partir de ejemplares de varias localidades de Japón y Corea, y una nueva especie del género, *Dracoderes gallaicus* sp. nov. procedente de las costas españolas. La nueva especie se distingue por la presencia de espinas laterales accesorias en el segmento 5. Además se proporcionan caracteres diagnósticos preliminares para una especie no descrita de la región de Okinawa, *Dracoderes sp.1*. También se propone una diagnosis enmendada para el género *Dracoderes* a partir de la nueva información sobre *D. abei* y *D. gallaicus*. El presente estudio incluye los primeros datos de microscopía electrónica de barrido para el género y presenta por primera vez información detallada sobre la morfología del introverto y la disposición de las escálidas así como nuevos datos sobre la disposición de las plácidas del cuello. También se demuestra la presencia de poros excretores (nefridioporos) especiales pero débilmente apreciables. La nueva información puede tener importancia filogenética y se espera que contribuya de forma importante a los resultados de futuros análisis filogenéticos sobre las relaciones dentro del filo Kinorrincos.

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ORIGINAL ARTICLE

On the genus *Dracoderes* Higgins & Shirayama, 1990 (Kinorhyncha: Cyclorhagida) with a redescription of its type species, *D. abei*, and a description of a new species from Spain

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Abstract

The type species of *Dracoderes*, *D. abei*, is redescribed based on specimens from several localities in Japan and Korea, and a new species of the genus, *Dracoderes gallaicus* sp. nov., is described from the coast of Spain in Western Europe. The new species is distinguished by the presence of lateral accessory spines on segment 5. In addition, preliminary diagnostic notes on a yet undescribed species from the Okinawa Region, *Dracoderes* sp. 1, are provided. Based on new information from *D. abei* and *D. gallaicus* sp. nov., an emended genus diagnosis for *Dracoderes* is proposed. The study includes the first scanning electron microscopical data for species of *Dracoderes*, and presents for the first time detailed information about head morphology and scalid arrangement, new data about the arrangement of the neck placids, and demonstrates the presence of feebly visible nephridial pores. The new information may be of phylogenetic significance, and is expected to contribute important data for future phylogenetic analyses of the kinorhynch interrelationships.

Key words: *Kinorhyncha, meiofauna, morphology, taxonomy*

Introduction

Kinorhyncha is a clade of marine, meiobenthic animals (Adrianov & Malakhov 1999; Sørensen & Pardos 2008). Currently, it accommodates 21 genera, some of which appear to have a global distribution, e.g. *Echinoderes*, *Campyloderes*, *Pycnophyes*, *Kinorhynchus* (Higgins 1983; Adrianov & Malakhov 1999; Neuhaus 2004). Conversely, some genera are currently monotypic and only known from their type localities, e.g. *Polacanthoderes*, *Triodontoderes*, *Tubulideres* and *Wollunquaderes* (see Sørensen et al. 2007; Sørensen 2008; Sørensen & Rho 2009; Sørensen & Thormar 2010), whereas others accommodate more than one species but still show a restricted geographic distribution on the generic level. The latter includes the genus *Fissuroderes* with five species, but with a known distribution restricted to the South Pacific (Neuhaus & Blasche 2006), *Neocentrophyes* with two species, restricted to the Indian Ocean (Higgins 1969), and *Dracoderes* with two species that are known from Korean–Japanese waters only (see Higgins & Shirayama 1990; Adrianov & Malakhov 1999).

Dracoderes was described by Higgins & Shirayama (1990), and species of the genus are easily recognized by the position of their dorsal spines that have been shifted from the usual middorsal position and instead alternatingly occur to the left and to the right of the middorsal line. The type species of the genus, *D. abei*, was first discovered from subtidal sandy mud in Mukaishima Yacht Harbor in the Inland Sea of Japan (Figure 1B), about 75 km east of Hiroshima (Higgins & Shirayama 1990). The second, and only other known species of the genus, *Dracoderes*

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orientalis, was described by Adrianov (1999) in Adrianov and Malakhov (1999) from Ulsan in the south-eastern part of Korea (Figure 1B), about 370 km from the type locality of *Dracoderes abei* Higgins & Shirayama, 1990. The two species are mostly discriminated by differing positions of tiny, feebly visible tubules and differences in the dimensions of body segments. The easiest way to distinguish between the species is by the male morphology, because males of *D. abei*, according to the original description, would have a middorsal spine on segment 10. Such a spine is missing in females of *D. abei* as well as in both sexes of *D. orientalis*.

During the Second International Scalidophora Workshop in 2007, organized by Y. Shirayama at the Seto Marine Biological Laboratory in Shirahama, about 200 km south-west of Mukaishima, some of the authors had the opportunity to collect specimens of *Dracoderes* at localities near the laboratory. The specimens were expected to be conspecific with *D. abei*, but they differed with minor details and by the absence of middorsal spine on segment 10 in males. Within the following couple of years, the authors obtained additional *Dracoderes* sp. from various localities off the Korean south coast and in the East China Sea. All these specimens were identical with those collected at Shirahama. Recently, additional specimens of *Dracoderes* were collected at different localities in the Inland Sea of Japan; some as close as 16 km from the type locality of *D. abei*, and again the specimens differed from *D. abei*, whereas they were identical with the previously collected specimens from Japan and Korea. These findings strongly indicated that the widespread Japanese/Korean species actually is conspecific with *D. abei* and that the original description of the species included some irregularities. Re-examination of the male allotype of *D. abei* confirmed this suspicion, and hence prompted a redescription of the species, which is included in the present contribution.

While specimens of *Dracoderes* were collected from East Asia, a new species of the genus also appeared in samples from Spain (Figure 1A). This is the first recording of the genus outside Asia, and the specimens differed from the two currently known species on important points. Hence, based on collected material from the two opposite sides of the Eurasian continent, we are able to present a redescription of *D. abei* and the description of a new, European species of *Dracoderes*.

Materials and methods

Specimens of *Dracoderes* spp. were collected at several localities in East Asia and Southwest Europe (Figure 1). Information on the localities, collecting data and recorded kinorhynch species is summarized in Table I. After sampling, meiofauna was extracted from the samples using the Ludox flotation method (Burgess 2001) or the bubbling and blot method (Higgins 1964; Sørensen & Pardos 2008), and the concentrated samples were subsequently fixed in formalin or preserved in ethanol (see Table I for exact concentrations and sample processing).

The fixed samples were rinsed with water to remove formalin and ethanol, and kinorhynch specimens (some stained with Rose Bengal) were picked up under a dissecting microscope. Specimens for light microscopy (LM) were dehydrated through a graded series of glycerin and mounted on a glass slide in Fluoromount G® or Hoyer's

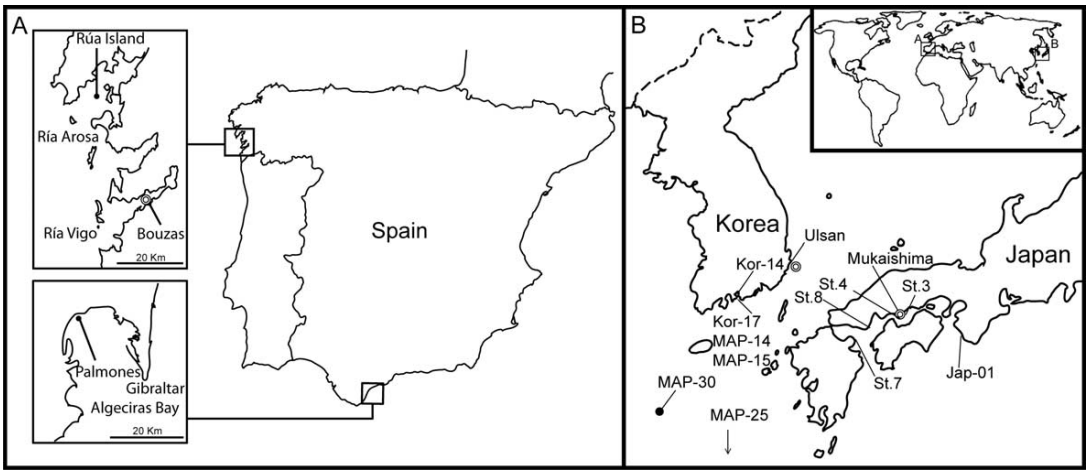

Figure 1. Map showing collecting localities in (A) Spain, with the Vigo and Algeciras areas shown as close-up in the inset; and (B) Korea and Japan. The type localities of *Dracoderes abei*, *D. orientalis* and *D. gallaicus* sp. nov. are marked with double circles. Please note that the locality MAP-25 in the East China Sea is located outside the range of the map.

Table I. Summary of data on collecting localities, methods, specimen processing and deposition. Abbreviations: ISJ, Inland Sea of Japan; NHMD, Natural History Museum of Denmark.

Station name	Location	Date	Position	Depth (m)	Sediment type	Collecting gear	Sample processing	Fixation	Kinorhynchs recorded	Collector	Deposition of specimens
JAP-01	Near Seto Marine Lab., Shirahama, ISJ	13 March 2007	33°42′25″N 135°23′00″E	14	Sandy mud	Smith–Macintyre grab	Bubbling and blot	4% formalin	*Dracoderes abei*, *Echinoderes* sp., *Kinorhynchus yushini*, *Pycnophyes tubuliferus*	MVS + FP	NHMD, Acc. No.: ZMUC KIN 491-496, MVS personal collection
St. 3	Sanagi-shima-Kita, ISJ, 36 km east of Mukaishima	27 October 2009	34°21′52″N 133°36′38″E	23–25	Mud	Dredge	Bubbling and blot	99% ethanol	*Dracoderes abei*, *Echinoderes* sp., *Kinorhynchus yushini*, *Pycnophyes* sp., *P. tubuliferus*	HY	HY pers. coll., Acc. No. ZIHU-03987
St. 4	Wakasu, ISJ, 16 km south of Mukaishima	27 October 2009	34°12′60″N 133°11′52″E	20	Mud	Smith–Macintyre grab	Bubbling and blot	99% ethanol	*Dracoderes abei*, *Echinoderes* sp.	HY	HY pers. coll., Acc. No. ZIHU-03988 to 03989
St. 7	Beppu wan Higasji, ISJ, 160 km SW of Mukaishima	29 October 2009	33°26′05″N 131°47′93″E	44	Mud	Smith–Macintyre grab	Bubbling and blot	99% ethanol	*Dracoderes abei*, *Echinoderes* sp., *Pycnophyes* sp.	HY	HY pers. coll., Acc. No. ZIHU-03990
St. 8	Yashirojima Nishi, ISJ, 110 km SW of Mukaishima	29 October 2009	33°54′24″N 132°09′27″E	17	Mud	Smith–Macintyre grab	Bubbling and blot	99% ethanol	*Dracoderes abei*, *Echinoderes* spp., *Kinorhynchus yushini*, *Pycnophyes* sp.	HY	HY pers. coll., Acc. No. ZIHU-03991
KOR-14	Gamak Bay, Korean south coast	28 May 2009	34°41′35″N 127°41′30″E	4.7	Mud	Smith–Macintyre grab	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Kinorhynchus* sp., *Pycnophyes* sp.	HSR + WM	NHMD, Acc. No.: ZMUC KIN 497-501, MVS & HSR personal collection
KOR-17	Gamak Bay, Korean south coast	28 May 2009	34°41′35″N 127°44′05″E	8	Mud	Smith–Macintyre grab	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Echinoderes rex*	HSR + WM	MVS personal collection
MAP-14	South of Gamak Bay, Korean south coast	26 May 2008	34°34′27″N 127°44′34″E	16	Mud	Smith–Macintyre grab	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Echinoderes* sp. 1, *E. rex*, *Kinorhynchus* sp.	HSR + WM	MVS personal collection

Table I (*Continued*)

Station name	Location	Date	Position	Depth (m)	Sediment type	Collecting gear	Sample processing	Fixation	Kinorhynchs recorded	Collector	Deposition of specimens
MAP-15	South of Gamak Bay, Korean south coast	26 May 2008	34°34′43″N 127°45′52″E	13	Mud with shells	Smith–Macintyre grab	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Echinoderes* sp. 2, *E. tchefouensis*, *Kinorhynchus* sp., *Pycnophyes* sp.	HSR + WM	NHMD, Acc. No.: ZMUC KIN 502–508, MVS & HSR personal collection
MAP-25	East China Sea	6 August 2008	28°32′69″N 125°09′53″E	104	Mud	Box corer	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Condyloderes* sp., *Echinoderes* sp., *Pycnophyes* sp.	HSR	MVS personal collection
MAP-30	East China Sea	6 October 2008	32°07′66 ″N 125°13′06″E	58	Mud with shells	Box corer	Ludox + centrifugation	4% formalin	*Dracoderes abei*, *Pycnophyes* sp.	HSR	MVS personal collection
Rúa Island 09.04.24.2	Ría de Arosa, Spain NW coast	24 April 2009	42°33′24″N 008°56′36″W	28	Mud	Higgins dredge	Bubbling and blot	7% formalin	*Dracoderes gallaicus* sp. nov., *Echinoderes* sp. 2, *E. cantabricus*	MH + NS + FP	NHMD, Acc. No.: ZMUC KIN 487-489, FP personal collection
Bouzas 09.04.25.6 09.09.25.5	Ría de Vigo, Spain NW coast	25 April and 25 September 2009	42°14′09″N 008°45′56″W	21	Mud	Higgins dredge	Bubbling and blot	7% formalin	*Dracoderes gallaicus* sp. nov., *E. cantabricus*, *Pycnophyes aulacodes*	MH + NS + FP + MVS	NHMD, Acc. No.: ZMUC KIN 490, FP personal collection
11.02.08.2 Algeciras Bay	Algeciras Bay, Spain SE coast	2 February 2011	36°10′35″N 005°26′46″W	16	Mud	Higgins dredge	Bubbling and blot	7% formalin	*Dracoderes gallaicus* sp. nov., *E. cantabricus*, *Kinorhynchus* sp., *Pycnophyes dentatus*	MH + NS + FP	FP personal collection
FP2069	Ishigaki Island, Ryukyu Islands, Japan	24 March 1986	24°20′63″N 124°08′42″E	3	Brown mud	Higgins dredge	?	?	*Dracoderes* sp. 1	Higgins & Shirayama	NHMD, Acc. No.: ZMUC KIN 509-521

medium. The mounted specimens were examined and photographed using an Olympus BX51 microscope equipped with an Olympus DP 20 or DP 70 camera. Measurements were made with Cell^A or Cell^D software. Specimens for scanning electron microscopy (SEM) were dehydrated through a graded series of alcohol, transferred to acetone and critical point-dried. Subsequently, the specimens were mounted on aluminium stubs, sputter coated with gold or a platinum/palladium mix and examined with either a JSM-6330F or a JSM-6335F JEOL SEM. Some specimens from the Korean samples were ultrasonically cleaned by exposing them to ultrasound for 20–25 s before the start of the dehydration procedure.

For comparison, the male allotype (RH2079.1/USNM235447) of *Dracoderes abei* was borrowed from the United States National Museum of Natural History (USNM). In an attempt to find additional specimens originating from the same samples as the *D. abei* type material, vials with unmounted kinorhynchs from the type locality and nearby stations were borrowed from USNM (RH2079-60, RH2079-63, RH2086/USNM860417.2, and RH2080/USNM860417.3). However, after inspection of all vials, it turned out that they only contained specimens of *Kinorhynchus yushini* (Adrianov, 1989). Additional material used in this study also includes specimens of a yet undescribed species of *Dracoderes* collected in Kabira Cove on Ishigaki Island in the Ryukyu Islands (FP2069, position 24°20′63″N 124°08′42″E), near Okinawa, Japan. The specimens originally belonged to the personal collection of R. P. Higgins but were subsequently donated to F. Pardos, who deposited them at the Natural History Museum of Denmark (accession numbers ZMUC KIN-509 to 521). They are not in a condition that allows a formal description, but they still provide information about the intrageneric variation within *Dracoderes*.

The following terminology generally follows Pardos et al. (1998), Neuhaus & Higgins (2002) and Sørensen & Pardos (2008). Furthermore, the term perispinal (most often used in taxonomic accounts for homalorhagid species) will be used for sensory spots flanking the laterally displaced spines in the dorsal series. These sensory spots would usually be referred to as paradorsal (or occasionally subdorsal) if flanking a middorsal spine, but this position is obscured by the lateral displacement of the spine to which they are associated.

Taxonomy

Cyclorhagida Zelinka, 1896
Dracoderidae Higgins & Shirayama, 1990
Dracoderes Higgins & Shirayama, 1990

Emended diagnosis for genus

Mouth cone with nine outer oral styles, alternating in size between larger and smaller ones. Neck with nine placids; dorsal and midlateral placids well-spaced, interrupted by cuticular foldings. Trunk segment 1 composed of closed cuticular ring; segments 2–11 of one tergal and two sternal plates. Dorsal spines on segments 2–9; spines on segments 2 and 9 in middorsal position, spines on segments 3–8 alternatingly laterally displaced left or right to paradorsal positions. Lateroventral spines on segments 6–9. Segment 11 with lateral terminal spines; females without lateral terminal accessory spines; males with 3 pairs of penile spines.

Dracoderes abei Higgins & Shirayama, 1990

Material examined

Male allotype, collected by R. P. Higgins and Y. Shirayama on 17 April 1986 in the Mukaishima Yacht Harbor, Inland Sea of Japan: 34°21′5″N, 133°13′0″E, 10 m depth, mounted in Hoyer's medium, stored at the USNM under accession number RH2079.1/USNM235447. Five specimens (one male and four females), mounted for LM, from 4 additional localities in the Inland Sea of Japan; 11 specimens (6 females, 3 males, 2 juveniles, and an adult for which the sex could not be determined), mounted for LM and SEM, from Shirahama in the eastern part of Inland Sea of Japan; numerous specimens (both sexes but with a majority of females), mounted for LM and SEM, from Gamak Bay, Korea; and 3 specimens (2 females and 1male), mounted for SEM, from the East China Sea. See Figure 1B for sampling localities and Table I for further details on localities, sampling, processing and deposition of specimens.

Emended diagnosis

Dracoderes with lateroventral tubules on segments 2, 5 and 10, and lateral accessory tubules on segment 8.

Description

Adult specimens with head, neck and 11 trunk segments (Figure 2A, B, 3A–C and 5A). See Table II for measurements and dimensions, and Table III for summary of spine, tubule and sensory spot locations.

Head and neck. Head consists of mouth cone and introvert (Figure 3 D–G), and an internal pharyngeal bulb. Pharyngeal bulb terminates in a pharyngeal

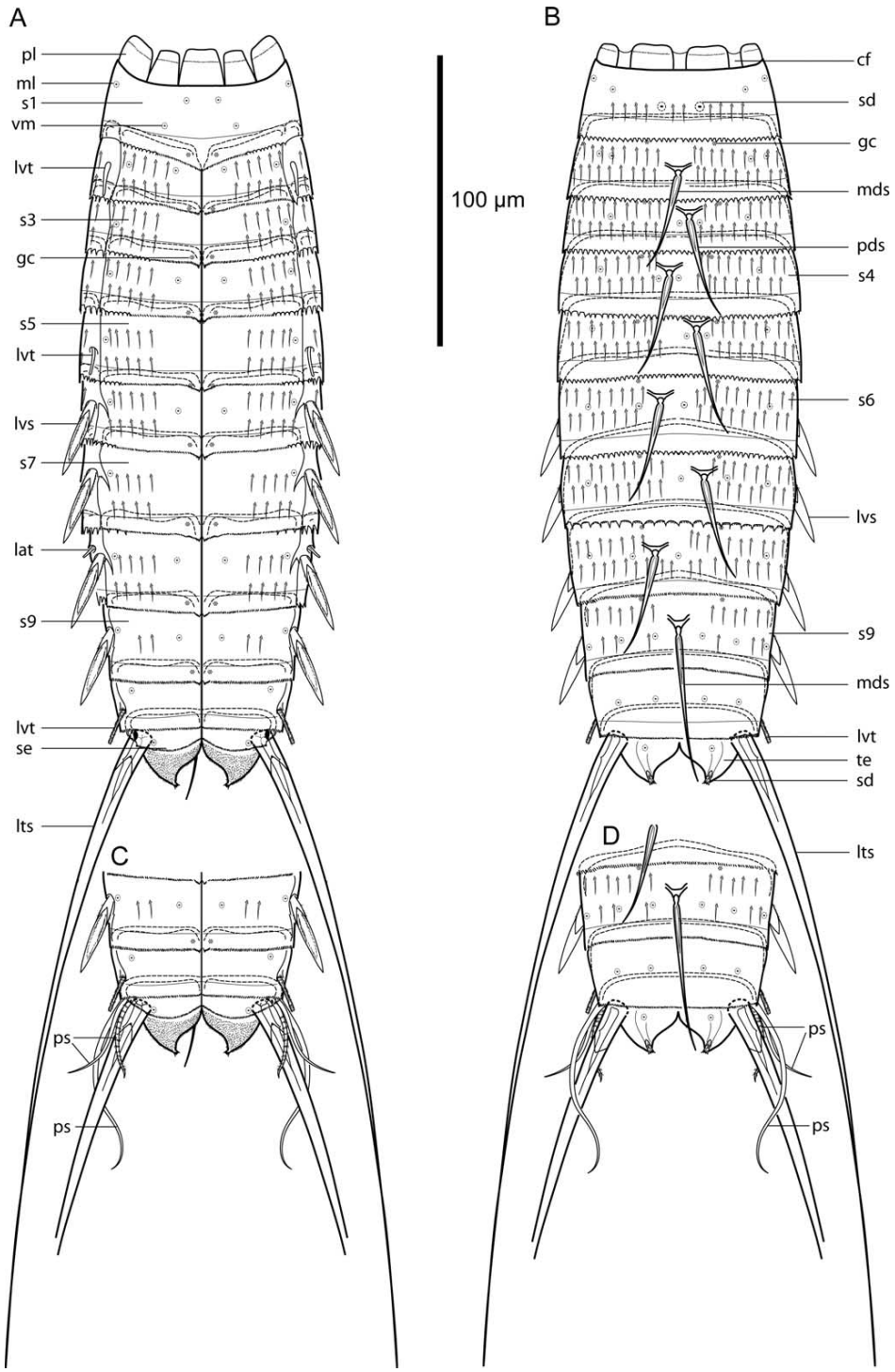

Figure 2. Line art illustrations of *Dracoderes abei*. (A) Female, ventral view; (B) female, dorsal view; (C) male, segments 9–11, ventral view; (D) male, segments 9–11, dorsal view. Abbreviations: cf, cuticular folding between placids; gc, glandular cell; lat, lateral accessory tubule; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; ml, midlateral sensory spot; pds, paradorsal spine; pl, placid; ps, penile spine; sd, subdorsal sensory spot; s, segment followed by segment number; se, sternal extension; te, tergal extension; vm, ventromedial sensory spot.

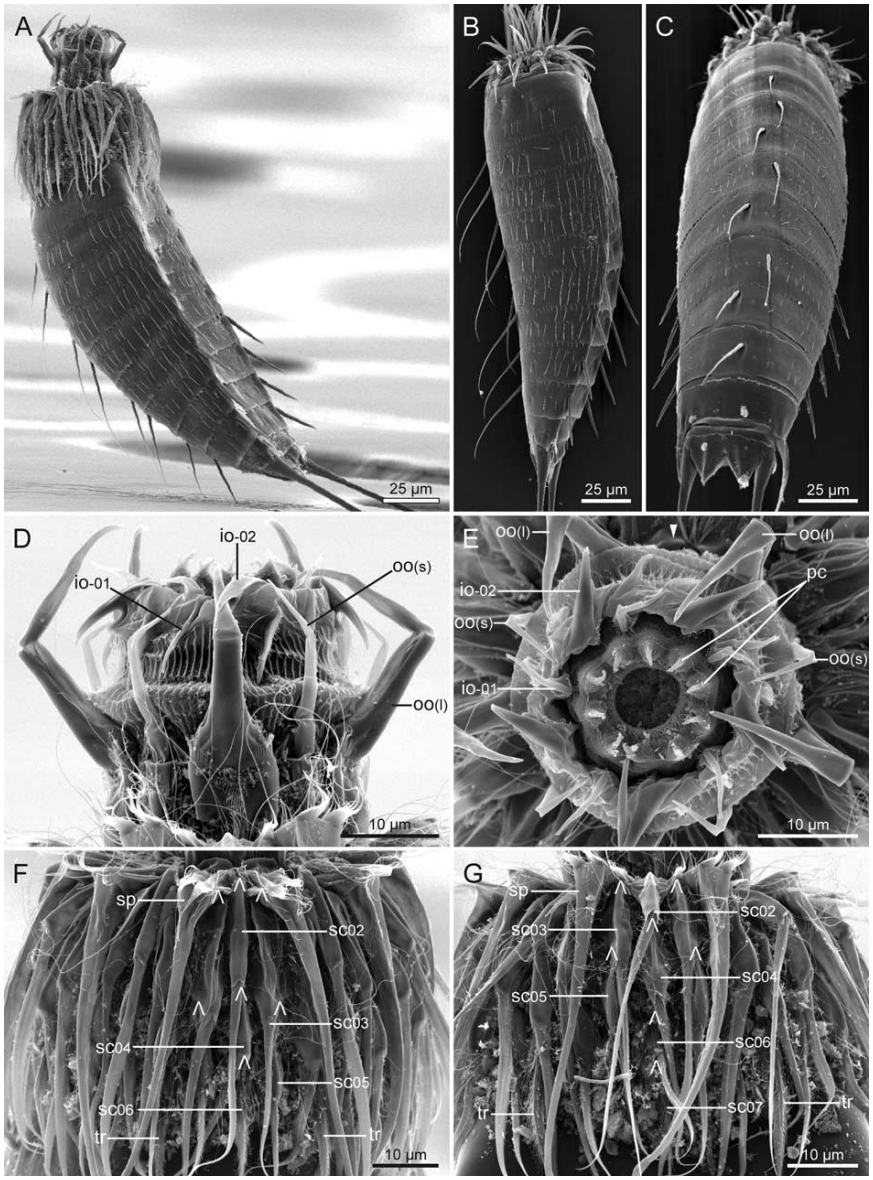

Figure 3. Scanning electron micrographs showing trunk overviews and details in the head of *Dracoderes abei*. (A) Lateroventral overview; (B) midlateral overview; (C) dorsal overview; (D) mouth cone, lateral view; (E) mouth cone, frontal view, arrowhead marks the position of the missing middorsal outer oral style; (F) introvert section 6 (middorsal section); (G) introvert section 1 (midventral section). Abbreviations: io, inner oral style; oo (s/l), outer oral style (small/large); pc, pharyngeal crown; sc, scalid; sp, spinoscalid; tr, trichoscalid. Digits after the labels refer to the mouth cone/introvert ring numbers. Lambda symbols λ mark attachment point of scalids.

crown formed by 10 tufts of cuticularized microvilli (Figure 3E). Internal part of mouth cone with three rings of inner oral styles. Anteriormost ring, Ring −03, with 5 inner oral styles consisting of a smooth, tubular end piece and a proximal, fringed sheath. Median ring, Ring −02, with 5 inner oral styles, forming large and well-developed helioscalids (Figure 3D,E). Posteriormost ring, Ring –01, with 10 inner oral styles, formed by smooth end pieces with a longitudinal line of fibrillae. External part of mouth cone with 9 outer oral styles (Figure 3D,E and 4). Outer oral styles alternate in size between larger and smaller ones (Figure 3D). Five large styles appear anterior to the odd numbered introvert sections, whereas four smaller ones appear anterior to the even numbered ones, except in the middorsal section 6 where a style is missing (Figure 4). Large styles consist of two joined subunits, and a basis with lateral pectinate fringes and a median area with bushy fringe tips. Smaller styles also consist of two subunits, but with their bases equipped with a median set of fringes that extend from an elevated

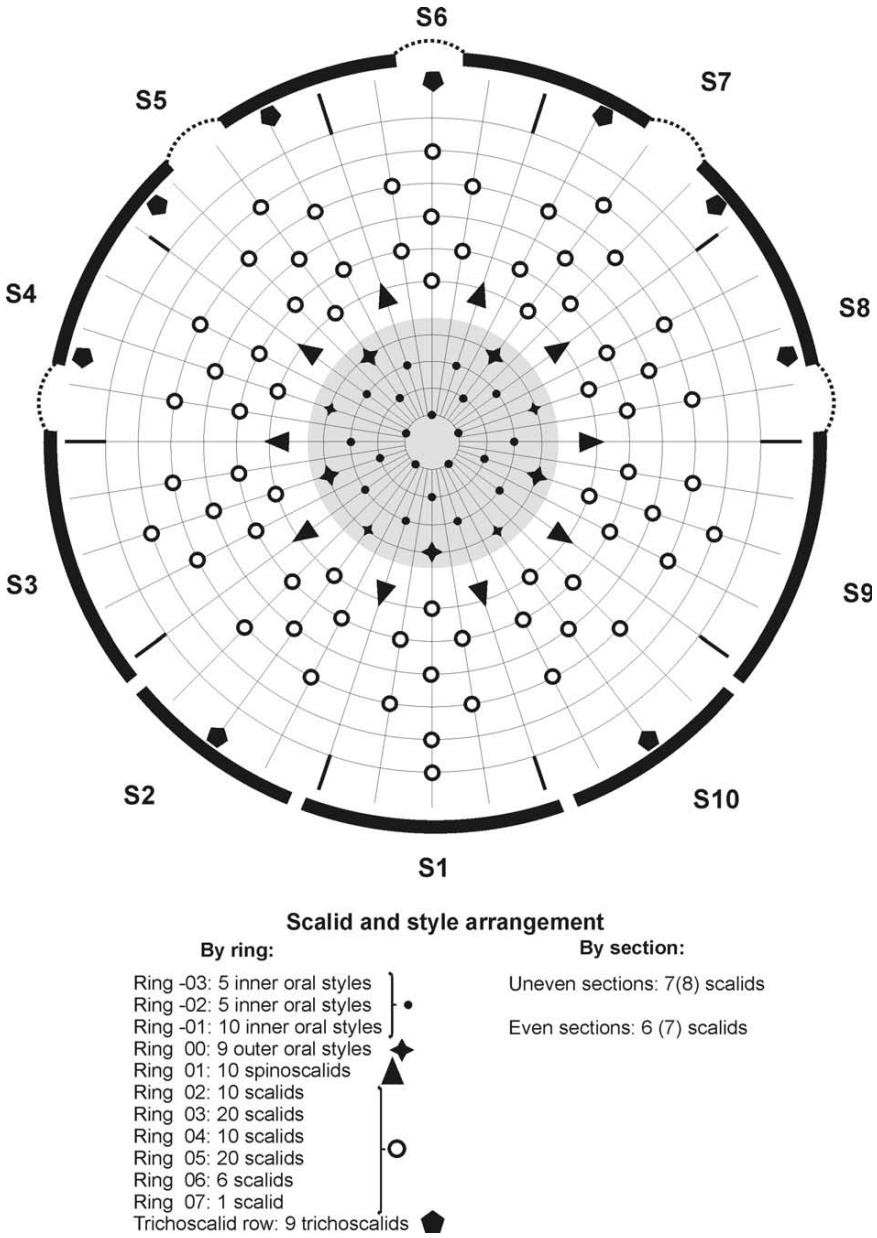

Figure 4. Diagram of mouth cone (grey shaded area), introvert and placids in *Dracoderes abei* with indication of inner and outer oral style, scalid and placid distribution. Placids are symbolized by the bent bars around the introvert diagram; cuticular foldings between placids are marked with punctuated lines.

lamella. All inner and outer oral styles have a terminal, slit-like pore.

Introvert with seven rings of scalids (Figure 4). Ring 01 with 10 spinoscalids consisting of long end piece and short basal sheath; posterior margin of sheath extends laterally into long, filiform fringes (Figure 3F). Ring 02 with 10 scalids, each consisting of a long end piece and a sheath with short marginal fringe. Rings 03 and 05 each with 20 scalids, two pr. introvert section, and Ring 04 with 10 scalids, representing the centre of the quincunx formed in each section by the Ring 03 to 05 scalids (Figure 4). Ring 06 with 6 scalids, present in section 6 and the five odd-numbered sections (Figure 3F, G and 4). Ring 07 with a single scalid only, present in section 1 (Figure 3G). Scalids in anterior rings resemble those in Ring 02, but become shorter towards the more posterior rings; posterior scalids furthermore with

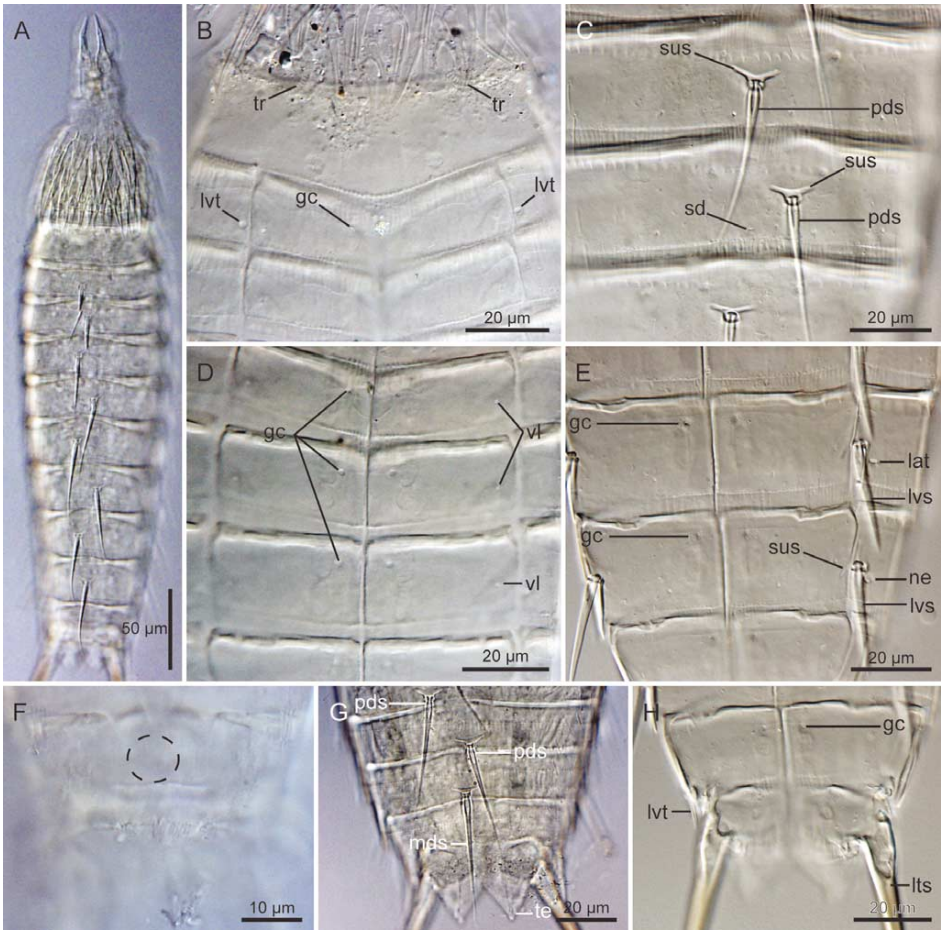

Figure 5. Light micrographs showing trunk overviews and details in the head of *Dracoderes abei*, including (B,D,F) male allotype (USNM235447), (A, G) specimen collected 16 km from type locality, and (C,E,H) specimen from locality KOR15 in Korea (ZMUC KIN-506). (A) Dorsal overview of head and trunk; (B) segments 1–3, ventral view; (C) segments 6–7, dorsal view; (D) segments 3–5, ventral view; (E) segments 8–9, ventral view; (F) segment 10 in male, focusing on middorsal position; note the absence of a middorsal spine in the marked area; (G) segments 8–11 in male, dorsal view; (H) segments 10–11 in female, ventral view. Abbreviations: gc, glandular cell; lat, lateral accessory tubule; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; ne, nephridiopore; pds, paradorsal spine; sd, subdorsal sensory spot; sus, subcuticular structure; te, tergal extension; tr, trichoscalid; vl, ventrolateral sensory spot.

fine serration on their lateral sides. Nine trichoscalids are present, and distributed as single ones in sections 2, 4, 6, 8, 10, and two in sections 5 and 7 (Figure 4).

The neck consists of 9 placids with a distinct joint between the neck and segment 1; widest placids are the midventral and midlateral ones, ca. 16 µm at base, whereas widths of the remaining ones vary between 11 and 14 µm. The 5 placids making up the paired midlateral and ventrolateral ones and the unpaired midventral one are situated closely together, whereas the midlateral, laterodorsal and subdorsal placids are well-spaced, with cuticular foldings occupying the space between the placids (Figures 4 and 6A). Each placid with transverse band of fine fringes, about a quarter of the distance from anterior margin (Figure 6A).

Trunk. The trunk has 11 segments, with segment 1 consisting of a closed cuticular ring, and segments 2–11 of one tergal and two sternal plates (Figure 2A, B, 3A–C and 5A, B). Midsternal junction visible as conspicuous line externally on the cuticle, whereas the tergosternal junctions are subcuticular, hence only visible with LM (e.g. compare Figure 5B and 6D). Tergal plates do not fold inwards towards the ventral side, but meet the sternal plates exactly in the lateroventral position (Figure 3A, B). In the anterior part of the trunk, the tergal plates are highly bulging dorsally, but flatten towards the posterior end, which

Table II. Measurements of adult *Dracoderes abei* from Japan and Korea and *Dracoderes gallaicus* sp. nov. from Spain, including number of measured specimens (*n*) and standard deviation (SD). There were no conspicuous differences in sizes or dimensions between the two sexes or populations. Abbreviations: ac, acicular spine; LA, lateral accessory; LTS, lateral terminal spine; LV, lateroventral spine/tubule; MD, middorsal spine; MSW-5, maximum sternal width (on segment 5); PD, paradorsal spine; S, segment lengths; SW-10, standard width (on segment 10); TL, trunk length; tu, tubule. N/A indicates that the measure is not applicable for the species.

	Dracoderes abei		*Dracoderes gallaicus* sp. nov.	
Character	Range	Mean (SD; *n*)	Range	Mean (SD; *n*)
TL (μm)	199–299	256 (31.27; 26)	182–245	210 (21.8; 7)
MSW-5 (μm)	65–79	72 (4.05; 26)	59–70	65 (3.63; 7)
MSW-5/TL (%)	22.7–36.0	28.9 (3.87; 25)	28.4–33.8	31 (2.08; 7)
SW-10 (μm)	51–63	57 (2.86; 25)	50–58	55 (2.54; 7)
SW-10/TL (%)	17.7–28.4	22.5 (3.44; 24)	23.9–28.7	26 (1.73; 7)
S1 (μm)	30–36	33 (1.64; 27)	32–39	35 (2.35; 6)
S2 (μm)	26–30	29 (1.07; 27)	27–30	28 (1.18; 6)
S3 (μm)	28–32	30 (1.07; 27)	27–29	28 (0.90; 6)
S4 (μm)	27–34	31 (1.37; 27)	28–32	30 (1.47; 6)
S5 (μm)	30–35	33 (1.34; 27)	31–37	33 (1.29; 6)
S6 (μm)	32–37	34 (1.37; 27)	31–34	33 (0.98; 6)
S7 (μm)	31–36	34 (1.34; 27)	32–34	33 (0.69; 6)
S8 (μm)	30–36	32 (1.49; 27)	32–34	32 (0.72; 5)
S9 (μm)	26–35	32 (1.84; 27)	31–33	32 (0.92; 6)
S10 (μm)	25–31	27 (1.31; 27)	22–32	27 (3.91; 6)
S11 (μm)	27–35	30 (1.85; 27)	32–35	33 (1.41; 6)
MD 2 (ac) (μm)	30–43	37 (3.63; 23)	32–35	34 (1.08; 6)
PD 3 (ac) (μm)	34–54	42 (5.78; 22)	33–43	39 (3.51; 7)
PD 4 (ac) (μm)	35–58	45 (6.19; 21)	41–47	44 (1.98; 7)
PD 5 (ac) (μm)	43–57	51 (3.86; 22)	43–52	45 (2.96; 7)
PD 6 (ac) (μm)	39–58	49 (5.17; 25)	41–47	44 (2.08; 7)
PD 7 (ac) (μm)	34–54	47 (4.26; 25)	38–48	43 (3.18; 7)
PD 8 (ac) (μm)	36–54	44 (5.33; 24)	38–45	41 (2.55; 7)
MD 9 (ac) (μm)	33–59	48 (5.45; 25)	41–58	50 (5.62; 7)
LV 2 (tu) (vm)	–	–	10–15	12 (1.86; 6)
LV 5 (tu) (μm)	–	–	12	12 (–; 1)
LA 5 (ac) (μm)	N/A	N/A	28–35	31 (2.42; 7)
LV 6 (ac) (μm)	25–30	27 (1.49; 27)	27–34	30 (2.68; 7)
LV 7 (ac) (μm)	22–31	27 (2.04; 27)	27–33	29 (1.94; 7)
LV 8 (ac) (μm)	24–29	26 (1.69; 27)	26–34	30 (2.50; 7)
LV 9 (ac) (μm)	24–31	27 (2.03; 27)	30–34	32 (1.84; 6)
LV 10 (tu) (μm)	7–11	8 (1.39; 8)	11–12	11 (0.70; 2)
LTS (μm)	230–291	252 (15.08; 25)	268–321	289 (18.59; 7)

gives the animal a characteristic tapering shape in lateral view (Figure 3B). Sternal plates reach their maximum width at segment 5, but almost maintain a constant width throughout the trunk. Generally, the sternal plates are wide, compared to the total trunk length (MSW-5:TL average ratio = 28.9%), which make the specimens appear rather plump.

Segment 1 without spines. One pair of sensory spots present in subdorsal and midlateral positions, and two pairs in laterodorsal and ventromedial positions (Figure 2A, B); sensory spots on this and all following segments are rounded with a collar of papillae around a central pore with a single sensory cilium (similar sensory spot from segment 7 exemplified on Figure 6B). A few, well-spaced cuticular hairs are present in a transverse row on the tergal plate; cuticular hairs on this and following segments appear flattened, and lanceolate rather than acicular, with indications of a median, longitudinal cleft; the trunk cuticle is slightly elevated at each hair perforation site, and the hairs emerge through posteriorly directed, slit-like openings in the cuticular elevations, showing a pair of tiny bracteae (Figure 6B). Posterior segment margin strongly serrated, extending midventrally forming a V-shaped extension and with rounded indentations without serrated edges in the lateroventral/ventrolateral positions (Figure 6D). A regular pectinate fringe is missing.

Segment 2 with middorsal spine (Figure 2B and 3C); dorsal spines on this and following segments are thin and acicular, composed of basal sheath with two deep incisions, and an acicular end piece with finely serrated margins; trunk cuticle around the attachment points of dorsal spines with conspicuous subcuticular structures that are best observed with LM (see Figure 5C for similar structures on segments 6 and 7). The basalmost part of the spine inside the segments cuticle is spherical, condyle-like,

Table III. Summary of nature and location of sensory spots, spines and tubules arranged by series in *Dracoderes abei* and *D. gallaicus* sp. nov. Abbreviations: LA, lateral accessory; LD, laterodorsal; LV, lateroventral; MD, middorsal; ML, midlateral; PD, paradorsal; PV, paraventral; SD, subdorsal; VL, ventrolateral; VM, ventromedial; ac, acicular spine; gc, glandular cell; lts, lateral terminal spine; m, male condition of sexually dimorphic character; ne, nephridiopore; pe, marks that the structure is perispinal and its exact position along the longitudinal series may have been obscured by eventual lateral spine displacement; ps, penile spine; ss: sensory spot; tu, tubule.

Dracoderes abei Position Segment	MD	PD	SD	LD	ML	LA	LV	VL	VM	PV
1			ss	ss ss	ss				ss ss	
2	ac		gc	ss ss			tu	ss	ss	gc
3		ac ss (pe)	gc	ss	ss			ss		gc
4		ac ss (pe)	gc	ss	ss			ss	ss	gc
5		ac ss (pe)	gc	ss	ss		tu	ss		gc
6		ac	ss (pe) gc	ss	ss		ac	ss	ss	gc
7		ac	ss (pe) gc	ss	ss		ac	ss		gc
8		ac	ss (pe) gc	ss		tu	ac	ss	ss	gc
9	ac		ss gc	ss	ss	ne	ac	ss	ss	gc
10			ss gc	ss			tu	ss		gc
11			ss ss				lts, psx3 (m)		ss	
Dracoderes gallaicus sp. nov.	MD	PD	SD	LD	ML	LA	LV	VL	VM	PV
1			ss	ss ss	ss				ss, ss	
2	ac	ss (pe)		ss ss			tu		ss	
3		ac ss (pe)		ss	ss			ss		
4		ac ss (pe)		ss	ss			ss	ss	
5		ac ss (pe)		ss	ss	ac	tu			
6		ac	ss (pe)	ss	ss		ac	ss	ss	
7		ac	ss (pe)	ss	ss		ac	ss		
8		ac	ss (pe)	ss		tu	ac	ss	ss	
9	ac		ss	ss	ss	ne	ac	ss	ss	
10			ss	ss			tu	ss		
11			ss, ss				lts psx3 (m)		ss	

suggesting a ball-and-socket articulation. Paired tubules present in the lateroventral positions; only the attachment points of tubules visible with LM (Figure 5B), whereas complete tubule is easily observed with SEM (Figure 6D); length estimated from SEM: 7 μm. Sensory spots present as twin pairs in laterodorsal position, and single pairs in ventrolateral and ventromedial positions (Figure 2A,B and 6D). Glandular cells present in subdorsal and paraventral positions (Figure 2A, B and 5B); cell outlets could not be identified with SEM. Cuticular hairs present in two transverse rows on both tergal and sternal plates; hairs in anterior rows shorter than those in the posterior ones (Figure 6D); segment otherwise smooth. A single secondary pectinate fringe encircles anteriormost part of segment. Posterior segment margin straight, with rough serration.

Segment 3 with a single dorsal spine, located in paradorsal position either to the left or to the right (Figure 2B and 3C); cuticular thickenings forming lateral extensions present around paradorsal spine on this and all following segments. Left or right displacement of the spine varies among specimens within populations, and there are no apparent left/right preference correlated with sex or sampling locality. Paradorsal spine with perispinal sensory spots; additional sensory spots located in laterodorsal, midlateral and ventrolateral positions (Figure 2A, B and 5D). Glandular cells, cuticular hairs, secondary pectinate fringes and posterior segment margin as on preceding segment.

Segment 4 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment (Figure 2B and 3C). Sensory spots as on preceding segment, with the addition of a ventromedial pair. Glandular cells, cuticular hairs, secondary pectinate fringes and posterior segment margin as on preceding segment.

Segment 5 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment. A pair of tubules present in the lateroventral positions (Figure 2A); only the attachment points of tubules visible with LM, whereas complete tubule is easily observed with SEM (Figure 6E); length estimated from SEM: 7 μm. Sensory spots, glandular cells, cuticular hairs, secondary pectinate fringe and posterior segment margin as on segment 3.

Segment 6 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment (Figure 2B, 3C and 5C). A pair of acicular spines present in the lateroventral positions; lateroventral spines are composed as the dorsal ones, with proximal ball, basal

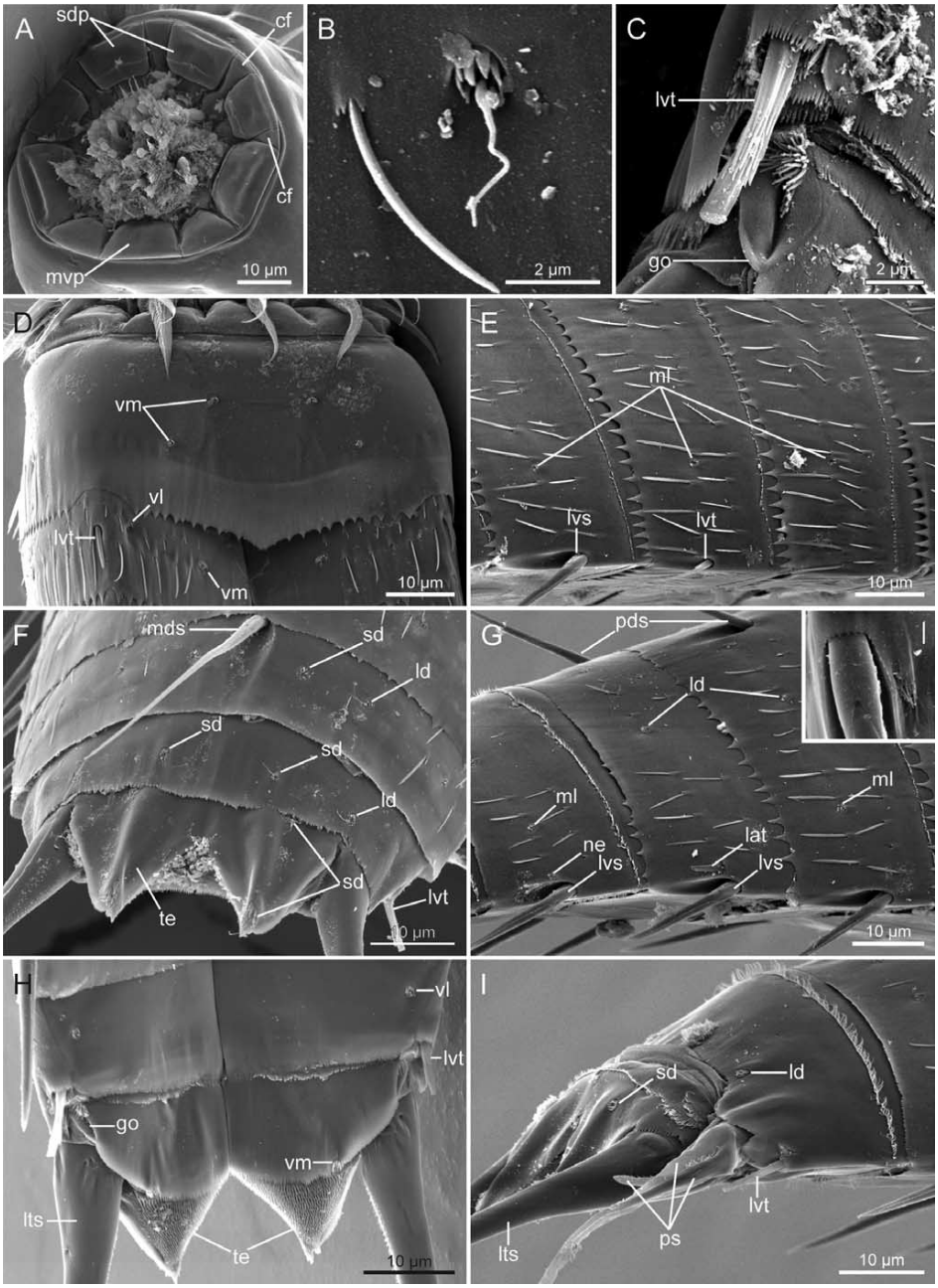

Figure 6. Scanning electron micrographs showing overviews and details in the cuticular trunk morphology of *Dracoderes abei*. (A) Frontal view of neck region in specimen with retracted head; (B) detail from segment 7 showing cuticular hair and subdorsal sensory spot; (C) detail from female specimen showing lateroventral tubule on segment 10 and gonopore on segment 11; (D) segments 1 and 2, ventral view; (E) segments 4–6, lateral view; (F) segments 9–11 in female specimen, dorsal view; (G) segments 7–9, lateral view; inset shows close-up of lateroventral spine basis and nephridiopore; scale in inset = 1 µm; (H) segments 10 and 11 in female specimen, dorsal view; (I) segments 10 and 11 in male specimen, lateral view. Abbreviations: cf, cuticular folding between placids; go, gonopore; lat, lateral accessory tubule; ld, laterodorsal sensory spot; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; ml, midlateral sensory spot; mvp; midventral placid; ne, nephridiopore; pds, paradorsal spine; ps, penile spine; sd, subdorsal sensory spot; sdp; subdorsal placid; te, tergal extension; vl, ventrolateral sensory spot; vm, ventromedial sensory spot.

sheath and acicular end piece, but lateroventral ones are much shorter and stouter, with blunt tips; trunk cuticle strongly enforced around the attachment point of the lateroventral spines, creating conspicuous subcuticular structures. Trunk cuticle around the attachment point of the spines with subcuticular thickenings and a ventrally directed extension; similar extensions present around lateroventral spines on all following segments (Figure 2A and 5E). Paradorsal spine with perispinal sensory spots, but

opposed to those on the preceding segment, the sensory spots here and on the following three segments are situated further away from the spine, in a position that would be subdorsal in specimens with middorsally aligned spines. Sensory spots otherwise present in laterodorsal, midlateral, ventrolateral and ventromedial positions. Glandular cells, cuticular hairs, secondary pectinate fringe and posterior segment margin as on preceding segment.

Segment 7 identical with preceding segment, except for the alternated lateral displacement of the dorsal spine, and the absence of ventromedial sensory spots.

Segment 8 identical to segment 6, except for the absence of midlateral sensory spots, and presence of lateral accessory tubules (Figure 2A, 5E and 6G); tubules are extremely small, ca. 4 μm, and their lateral position and the underlying subcuticular structures make them almost impossible to visualize with LM unless the specimen is squeezed and observed from a slightly lateral view (e.g. as on Figure 5E); tubules are easily observed with SEM. Paradorsal spine on this segment is often shorter than those on the four previous segments and the middorsal one on segment 9 (see Table II).

Segment 9 with dorsal and lateroventral spines; dorsal spine located in perfect middorsal position (Figure 2B, 3C, 5A, G and 6F). Sensory spots present in subdorsal, laterodorsal, midlateral, ventrolateral and ventromedial positions. Nephridial pores present in lateral accessory positions; pore not sieve-like, but formed by a minute, posteriorly directed opening with a few papillae (Figure 5E and 6G with inset). Only a few, or eventually no, cuticular hairs present. Posterior segment margin without rough serration, instead with very fine pectinate fringe (Figure 6F). Glandular cells and secondary pectinate fringe as on preceding segment.

Segment 10 without spines in both sexes (Figures 2 and 5F, G and 6F, I), but with pair of lateroventral tubules. Tubules thick and hairy, with collar of papillar extensions near distal tips (Figure 6C). Sensory spots present in subdorsal, laterodorsal, and ventrolateral positions (Figure 6F, H). Tergosternal junction straight, without incisions (Figure 5H). Cuticular hairs not present. Glandular cells, secondary pectinate fringe and posterior segment margin as on preceding segment.

Segment 11 with lateral terminal spines; lateral terminal accessory spines not present in females or males (Figures 2 and 5F and 6F). Females with gonopores present as slit-like openings on the anterolateral margins of the sternal plates (Figure 6H). Males with three pairs of penile spines; longest penile spine with very thick basis; second, slightly shorter penile spine attaches on the basis of the longest penile spine; third penile spine short, composed of small, fringed collars, giving it a moniliform or crenulated appearance (Figure 2C, D and 6I). Two pairs of subdorsal sensory spots present: one pair on anterior part of segment, the second on the tips of the tergal extensions (Figure 6F, I); sensory spots furthermore present in ventromedial positions on posterior margins of sternal plates (Figure 6H). Glandular cells not present. Cuticular hairs not present. Secondary pectinate fringe with slightly longer fringe tips, otherwise as on preceding segment. Posterior margin of tergal plate forms bulged, perfectly triangular tergal extensions, extending well beyond sternal plates; dorsal side of extensions smooth (Figure 6F), ventral side densely covered with short, papillar hairs (Figure 6H). Posterior margins of sternal plates slightly rounded, without any projecting parts (Figure 6H).

***Dracoderes gallaicus* sp. nov.**

Type material

Holotype, adult male, collected on 25 April 2009 at Bouzas, Ría de Vigo, near Vigo in Galicia, Spain: 42°14′09″N, 008°45′56″W (Figure 1A), at 21 m depth; mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark (NHMD), under accession number: ZMUC KIN-487. Allotype, adult female, same collecting data as holotype, mounted in Fluoromount G®, deposited at NHMD under accession number: ZMUC KIN-488. Paratypes, two adult males; one collected on 25 September 2009 at same locality as holotype, mounted in Fluoromount G®, deposited at NHMD under accession number: ZMUC KIN-489; second collected on 24 April 2009 near Rúa Island, Ría de Arosa, near Vigo in Galicia, Spain: 42°33′24′N, 008°56′36″W (Figure 1A), at 28 m depth; mounted in Fluoromount G®, deposited at NHMD under accession number: ZMUC KIN-490. Other specimens from the two localities (3 mounted for LM and 15 for SEM) are stored in the authors' collection at the Universidad Complutense, Madrid, Spain. Additional specimens of this species were collected on 8 February 2011, after finalizing the description of type specimens recorded from Algeciras Bay, west of Gibraltar, Spain: 36°10′35″N, 005°26′46″W (Figure 1A), at 16 m depth; mounted in Fluoromount G®, and stored in the authors' collection at the Universidad Complutense, Madrid, Spain.

Diagnosis

Dracoderes with lateroventral tubules on segments 2, 5 and 10, lateral accessory spines on segment 5 and lateral accessory tubules on segment 8.

Etymology

The species name is derived from Latin *gallaicus*, meaning 'from Gallaecia', the Roman name for Galicia, the region in NW Spain where the animals were found.

Description

Adult specimens with head, neck and 11 trunk segments (Figure 7A, B, 8A, B and 9A, B). See Table II for measurements and dimensions, and Table III for summary of spine, tubule and sensory spot locations.

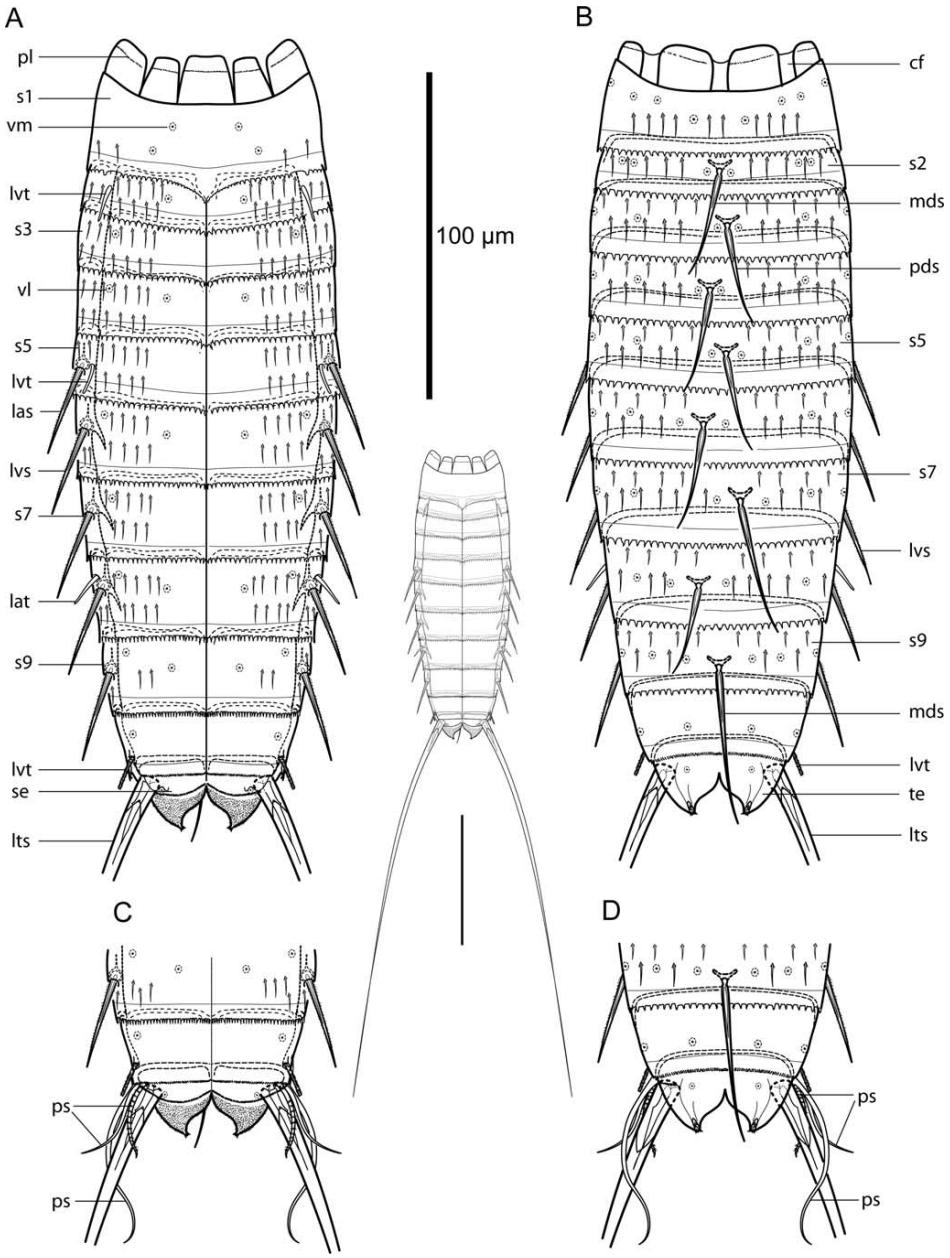

Figure 7. Line art illustrations of *Dracoderes gallaicus* sp. nov. (A) Female, ventral view; (B) female, dorsal view; (C) male, segments 9–11, ventral view; (D) male, segments 9–11, dorsal view. Inset in the centre shows a specimen with lateral terminal spines drawn to full length; scale = 100 µm. Abbreviations: cf, cuticular folding between placids; las, lateral accessory spine; lat, lateral accessory tubule; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; pds, paradorsal spine; pl, placid; ps, penile spine; s, segment followed by segment number; se, sternal extension; te, tergal extension; vl, ventrolateral sensory spot; vm, ventromedial sensory spot.

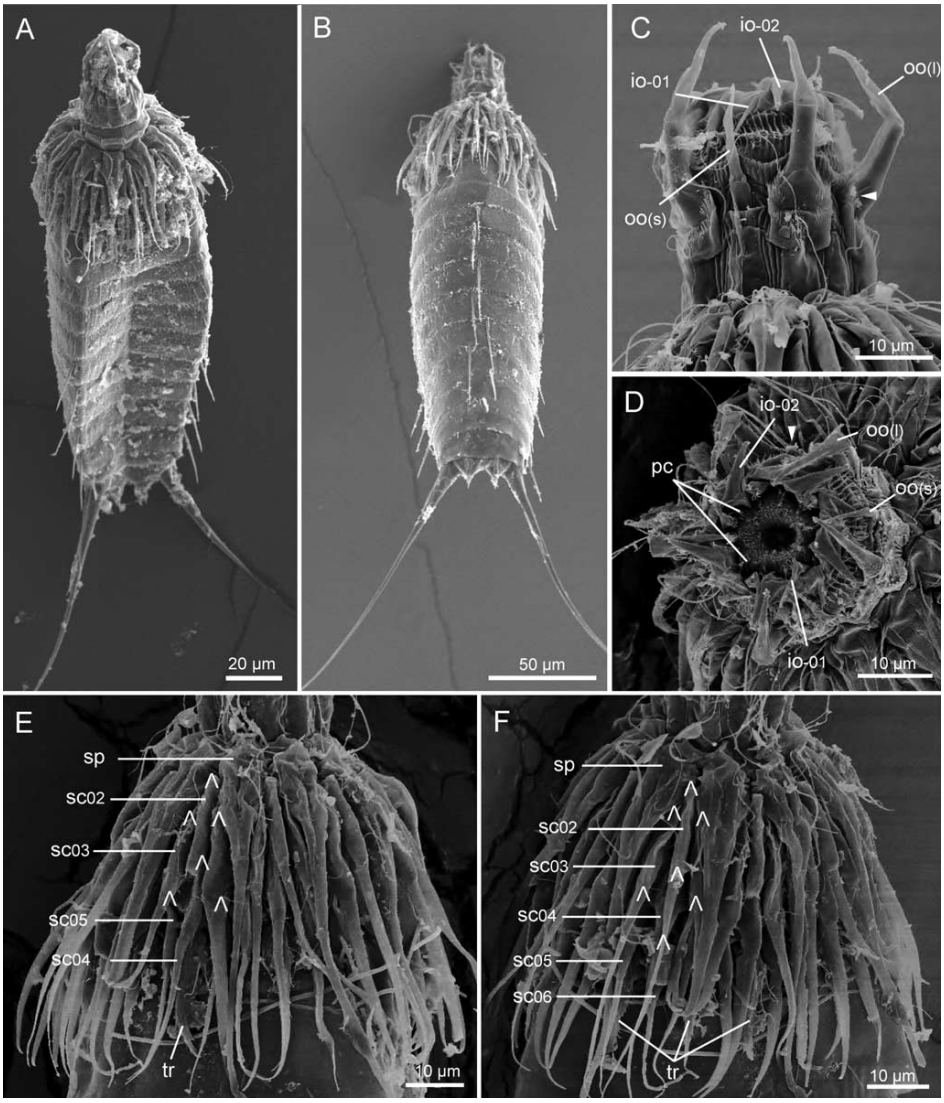

Figure 8. Scanning electron micrographs showing trunk overviews and details in the head of *Dracoderes gallaicus* sp. nov. (A) Ventrolateral overview; (B) dorsal overview; (C) mouth cone, laterodorsal view, arrow head marks the position of the missing middorsal outer oral style; (D) mouth cone, frontal view, arrow head marks the position of the missing middorsal outer oral style; (E) introvert section 4; (F) introvert section 5. Abbreviations: io, inner oral style; oo (s/l), outer oral style (small/large); pc, pharyngeal crown; sc, scalid; sp, spinoscalid; tr, trichoscalid. Digits after the labels refer to the mouth cone/introvert ring numbers. Lambda symbols λ mark attachment point of scalids.

Head and neck. Head consists of mouth cone and introvert, and an internal pharyngeal bulb. The internal pharyngeal bulb ends in a pharyngeal crown equipped with 10 minute tooth-like structures formed by cuticularized microvilli (Figure 8D) as described in *D. abei.* The mouth cone is formed by three rings of inner oral styles (Figure 8C). Anteriormost ring, Ring −03, with 5 inner oral styles; median ring, Ring –02, with 5 inner oral styles and posteriormost ring, Ring −01, with 10 inner oral styles. External part of mouth cone with 9 outer oral styles alternating in size between five larger and four smaller ones (Figure 8C,D). Details on morphology of oral styles as described for *D. abei*. Introvert with seven rings of scalids (Figure 8E,F). Scalid arrangement and morphology exactly the same as described for *D. abei* (see description above and Figure 4).

Neck with nine placids, four dorsal and five ventral. Dorsal and midlateral placids well-spaced with cuticular foldings in between (Figure 9E), as described for *D. abei* (Figure 3), whereas ventral placids are situated closely together. Widest placids are the midventral and midlateral ones, measuring 15 and 17 µm, respectively; widths of remaining ones vary between 12 and 14 µm.

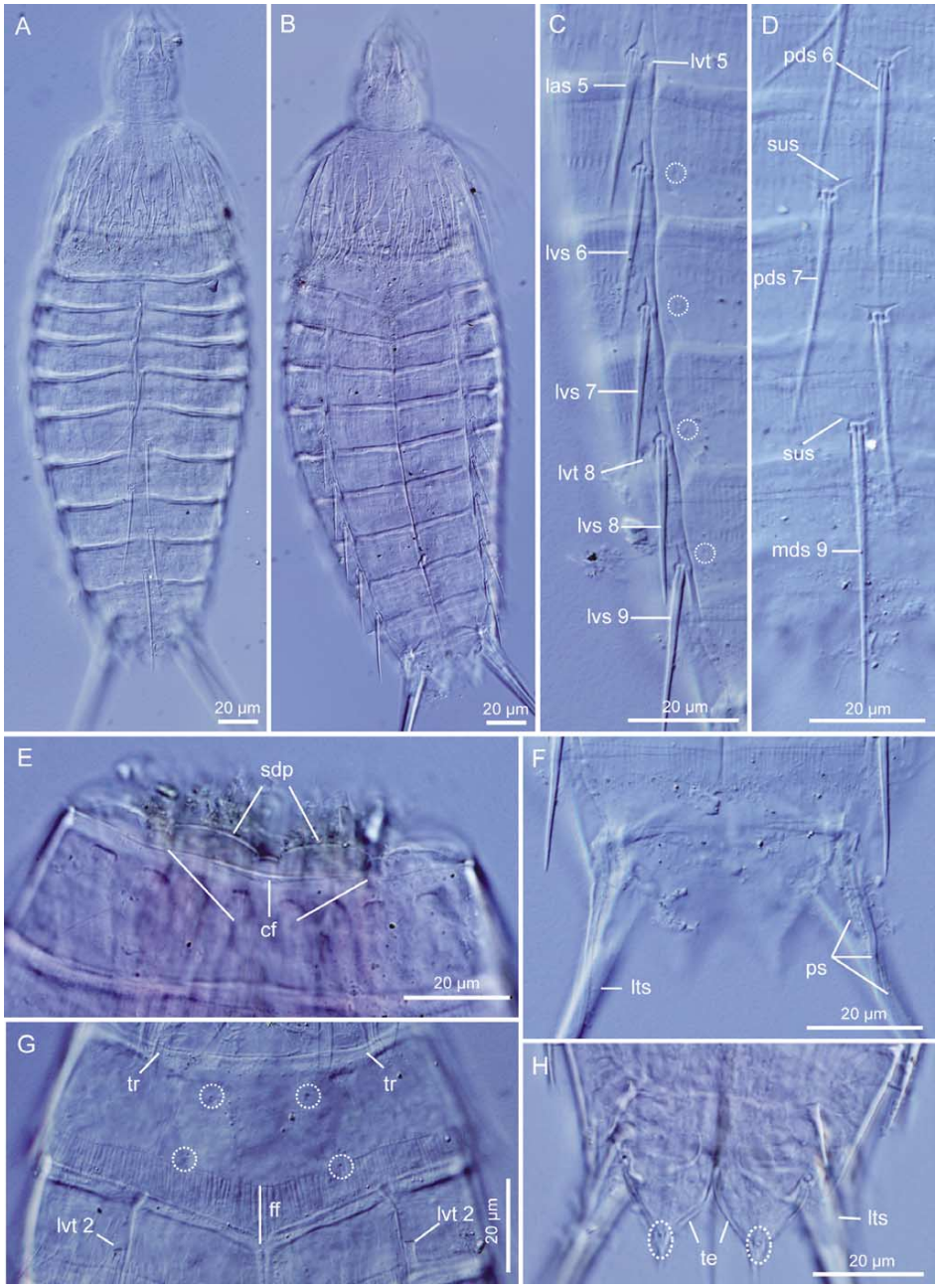

Figure 9. Light micrographs showing trunk overviews and details in trunk morphology of *Dracoderes gallaicus* sp. nov., including (A,C,D,F) male holotype (ZMUC KIN-487), (G) female allotype (ZMUC KIN-488), (B) male paratype (ZMUC KIN-489), and (E,H) two non-types. (A) Dorsal overview of head and trunk; (B) ventral overview of head and trunk; (C) lateroventral spines on segments 5–9; (D) middorsal spines on segments 6–9; (E) dorsal placids and segment 1; (F) segments 10–11 in male, ventral view; (G) segments 1–2, ventral view; (H) segments 10–11 in male, dorsal view. Abbreviations: cf, cuticular folding between placids; ff, free flap; las, lateral accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; pds, paradorsal spine; ps, penile spine; sdp, subdorsal placid; sus, subcuticular structure; te, tergal extension; tr, trichoscalid. Sensory spots are marked with punctuated circles. Digits after the labels refer to segment number.

Trunk. The trunk has 11 segments. Segment 1 consists of one closed cuticular ring, segments 2–11 of one tergal and two sternal plates (Figure 7A, B and 9A, B). Trunk shape and tergosternal junctions as in *D. abei.* Cuticle thick, with well developed pachycycli. Sternal plates wide compared to the total trunk length

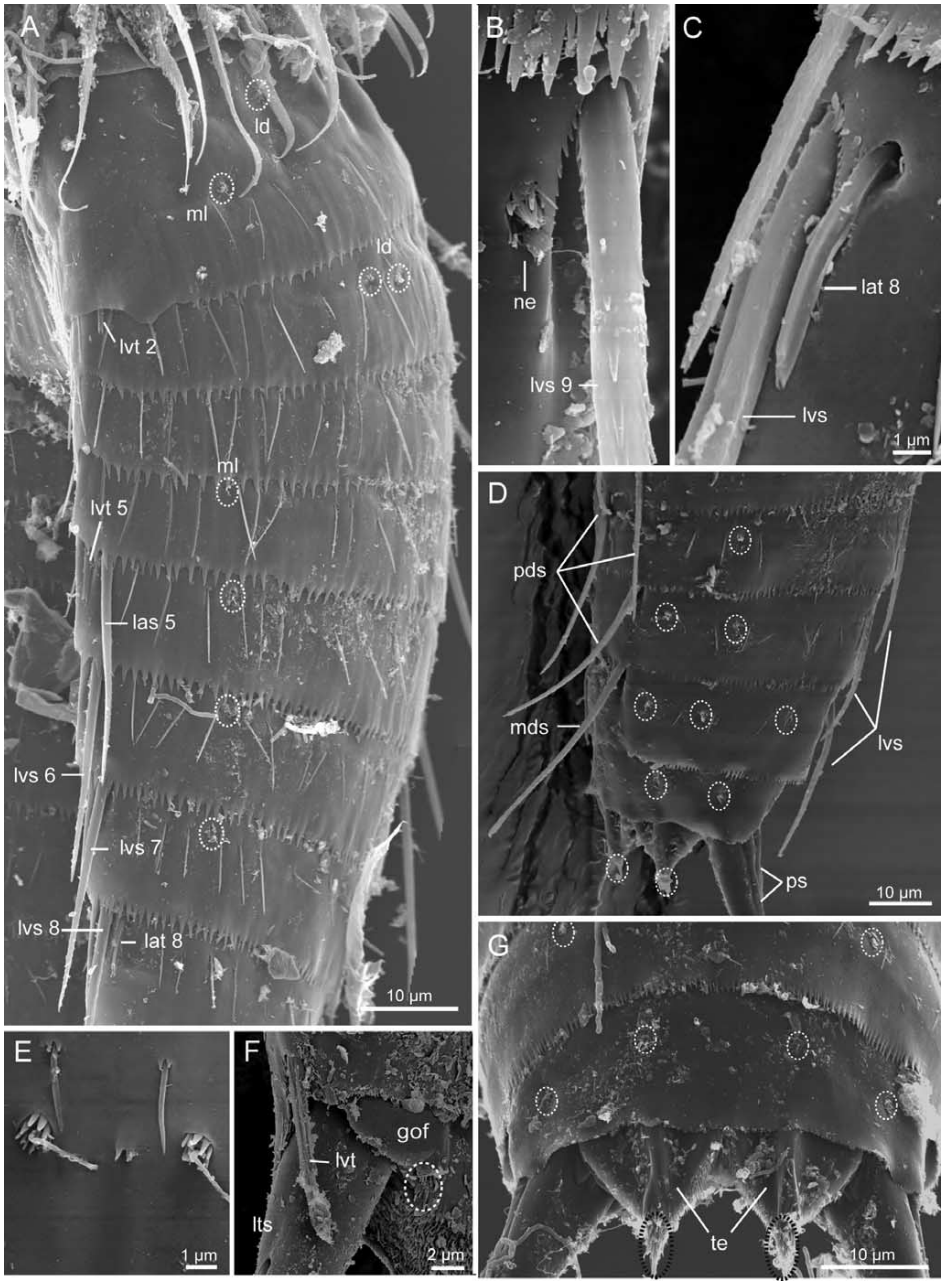

Figure 10. Scanning electron micrographs showing overviews and details in the cuticular trunk morphology of *Dracoderes gallaicus* sp. nov. (A) Segments 1–8, lateral view; (B) detail from segment 9 showing lateroventral spine basis and nephridiopore in lateral accessory position; (C) detail from segment 8 showing lateroventral spine basis and tubule in lateral accessory position; (D) segments 7–11 of male specimen, laterodorsal view; (E) detail showing laterodorsal sensory spots on segment 2; (F) detail from female specimen showing lateroventral tubule on segment 10 and gonopore flap on segment 11; (G) segments 9–11 of male specimen, dorsal view. Abbreviations: gof, gonopore flap; las, lateral accessory spine; lat, lateral accessory tubule; ld, laterodorsal sensory spot; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; ml, midlateral sensory spot; ne, nephridiopore; pds, paradorsal spine; ps, penile spine; te, tergal extension. Sensory spots are marked with punctuated circles. Digits after the labels refer to segment number.

(MSW-6:TL average ratio = 31%), reaching their maximum width at segment 6. Secondary pectinate fringe and glandular cells could not be observed.

Segment 1 without spines. Single pairs of sensory spots present in subdorsal and midlateral positions, and two pairs in laterodorsal and ventromedial position (Figure 7A, B, 9G and 10A); sensory spots on this and all following segments are rounded with collar of papillae around a central pore with a single sensory cilium (e.g. as on Figure 10E). Few,

well-spaced cuticular hairs emerging through rounded perforation sites without bracteae, present along a transverse row. Posterior part of segment midventrally extended into a conspicuous V shape, forming a wide, free flap (Figure 9G). Posterior segment margin strongly serrated, except in the lateroventral/ventrolateral areas which are slightly indented and smooth (Figure 10A).

Segment 2 with middorsal spine (Figure 7B, 8B and 9A); dorsal spines on this and following segments are thin and acicular, with same composition and subcuticular thickenings forming laterally directed extensions, as described from *D. abei* (Figure 9D). Paired tubules (7 μm) present in lateroventral positions (Figure 7A, 9G and 10A). Sensory spots present as twin pairs in laterodorsal position (Figure 10A,E), and single pairs in paradorsal and ventromedial positions. Cuticular hairs present in two transverse rows on both tergal and sternal plates; hairs in anterior row shorter than those in posterior row. Posterior segment margin serrated with alternating large and small teeth.

Segment 3 with a single dorsal spine, located in paradorsal position either to the left or to the right (Figure 7B). Left or right displacement of the spine varies among specimens within populations as explained for *D. abei*. Paradorsal spine with perispinal sensory spots; additional sensory spots located in laterodorsal, midlateral and ventrolateral positions. Cuticular hairs and posterior segment margin as on preceding segment.

Segment 4 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment. Sensory spots as on preceding segment, with the addition of a ventromedial pair. Cuticular hairs and posterior segment margin as on preceding segment.

Segment 5 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment. A pair of tubules present in the lateroventral positions (Figure 7A, 9C and 10A); only attachment points of tubules are visible with LM (Figure 9C), whereas complete tubules are easily observed with SEM (Figure 10A); length ca. 8 μm. A pair of acicular spines present in the lateral accessory position (Figure 7A, 9C and 10A), showing a conspicuous subcuticular mark only visible with LM in the form of a longitudinal slit directed anteriorly from the spine basis (Figure 9C); similar subcuticular structures present around lateroventral spines of the following four segments (Figure 9C). Paradorsal spine with perispinal sensory spots; additional sensory spots located in laterodorsal and midlateral positions (Figure 10A). Cuticular hairs and posterior segment margin as on preceding segment.

Segment 6 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment (Figure 7B and 9D). A pair of long, acicular spines present in the lateroventral positions (Figure 7A, 9C and 10A). Tergosternal junction with deep, posterioventrally directed incision. The paradorsal spine is flanked by perispinal sensory spots, but in a more subdorsal position than those on the preceding segment. Sensory spots otherwise present in laterodorsal, midlateral, ventrolateral and ventromedial positions (Figure 9C and 10A). Cuticular hairs and posterior segment margin as on preceding segment.

Segment 7 identical with preceding segment, except for the alternated lateral displacement of the dorsal spine, and the absence of ventromedial sensory spots.

Segment 8 with single dorsal spine, located in a paradorsal position opposite to the side of the dorsal spine on the preceding segment (Figure 7B and 9D). Paradorsal spine on this segment is often shorter than those on the four previous segments and the middorsal one on segment 9 (see Table II). Lateroventral spines and lateral accessory tubules (length 8 μm) present (Figure 7A, 9C and 10C). Tergosternal junction with deep, posterioventrally directed incision. Perispinal sensory spots present in subdorsal position; additional sensory spots present in laterodorsal (Figure 10D), ventrolateral and ventromedial positions (Figure 9C). Cuticular hairs and posterior segment margin as on preceding segment.

Segment 9 with dorsal and lateroventral spines; dorsal spine located in perfect middorsal position (Figure 7B, 9D and 10D). Tergosternal junction without posterioventrally directed incision. Sensory spots present in subdorsal, laterodorsal, midlateral, ventrolateral and ventromedial positions (Figure 10D). Nephridial pores present in lateral accessory positions; pore not sieve plate-like, formed by minute, posteriorly directed, opening surrounded by a few papilla (Figure 10B). Only few cuticular hairs present. Posterior segment margin as on preceding segment.

Segment 10 without spines, but with a pair of thick lateroventral tubules (length 12 μm) (Figure 7A,C and 10F), similar to those in *D. abei*. Sensory spots present in subdorsal, laterodorsal and ventrolateral positions (Figure 10D,G). Cuticular hairs absent. Posterior segment margin finely serrated with short and soft tips.

Segment 11 with long lateral terminal spines (see inset in Figure 7); lateral terminal accessory spines present neither in females nor males. Males with three pairs of penile spines (Figure 7C,D); longest penile spine (59 μm) very flexible (Figure 9F); second, slightly shorter one attaches on the basis of the longest penile spine; third penile spine short,

composed of small, fringed collars, giving it a crenulated appearance. Females with gonopores present on the anterolateral margins of the sternal plates; gonopore covered by an oval flap (Figure 10F). Two pairs of subdorsal sensory spots, one pair situated on anterior part of segment and the second pair on the tips of the tergal extensions (Figure 9H and 10D, G); additional sensory spots in ventromedial position on posterior margins of sternal plates (Figure 7A and 10F). Cuticular hairs not present. Posterior margin of tergal plate forms perfectly triangular tergal extensions, extending well beyond sternal plates; dorsal side of extensions smooth, ventral side densely covered with short, papillar hairs. Posterior margins of sternal plates slightly rounded.

***Dracoderes* sp. 1.**

The following is not a formal species description, but an attempt to provide preliminary morphological information about one additional, yet undescribed species of *Dracoderes*. The information should serve to (1) enable identification of the species if it gets recollected in the future, and (2) to add to our knowledge about morphological disparity within the genus.

Material examined

Thirteen specimens, collected by R.P. Higgins and Y. Shirayama on 23 March 1986 in Kabira Cove, Ishigaki Island near Okinawa, Japan: 24°20′63″N, 124°08′42″E, at 3 m depth; most probably mounted in Hoyer's medium, donated to FP, and stored in the NHMD under accession numbers ZMUC KIN-509 to KIN-521. Several specimens were in a poor condition and could not be examined in detail, either because of desiccation or crystallization of the mounting medium.

Description

The species generally follows the proposed emended diagnosis for the genus. Dorsal spines are present on segments 2–9, located in a middorsal position on segments 2 and 9, and alternatingly laterally displaced to paradorsal positions on segments in between (Figure 11A, B). All dorsal spines have the same subcuticular structures at their attachments points as those described from *D. abei* and *D. gallaicus* sp. nov., but the spines are conspicuously short and plump, hence resembling those in the lateroventral series. Lateroventral tubules present on segments 2 and 5 (Figure 11C, D), lateroventral spines on segments 6–9 (Figure 11D, E). Eventual presence of lateroventral tubules on segment 10 could not be determined. Lateral accessory tubules appear to be absent on segment 8, but confirmation from SEM would be desirable. Other noteworthy features include the posterior segment margin of segment 2 that form a strong pectinate fringe (Figure 11C), which appears much more distinct than the

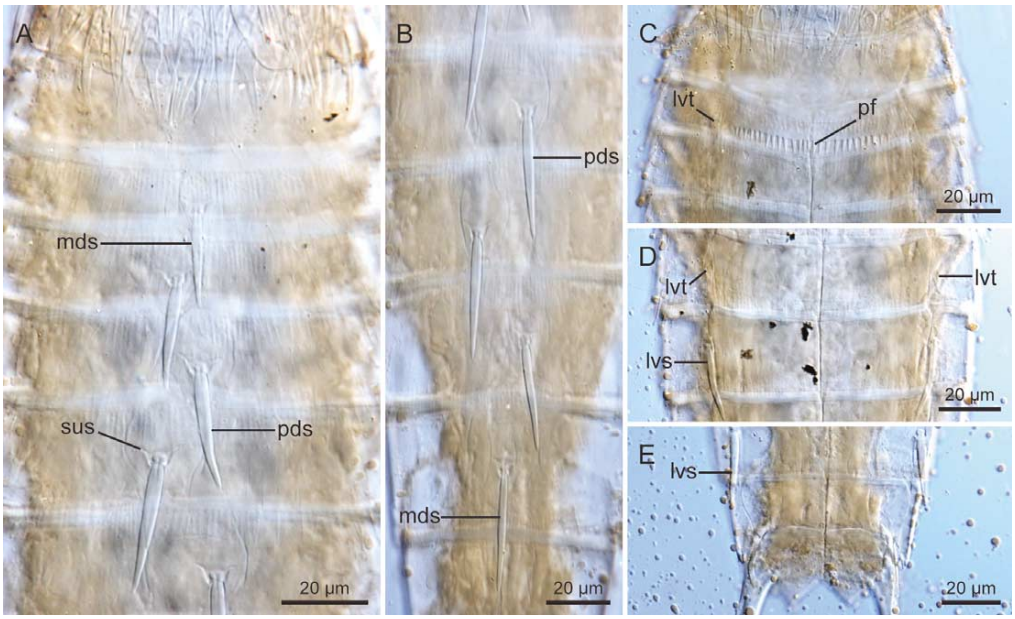

Figure 11. Light micrographs showing trunk overviews and details in trunk morphology of *Dracoderes* sp. 1 (ZMUC KIN-517) from the Okinawa region. (A) Segments 1–6, dorsal view; (B) segments 6–10, dorsal view; (C) segments 1–3, ventral view; (D) segments 5–6, ventral view; (E) segments 9–11 in male, ventral view. Abbreviations: lvs, lateroventral spine; lvt, lateroventral tubule; mds, middorsal spine; pds, paradorsal spine; pf, pectinate fringe; sus, subcuticular structure.

fine serration of posterior margins on other segments. Sensory spots and glandular cells could not be observed.

Discussion

Differential notes on species of Dracoderes

Currently, three valid species can be assigned to *Dracoderes*: *D. abei*, *D. orientalis* and the new species described herein, *D. gallaicus* sp. nov. The three species are most easily discriminated by the presence/absence of tubules and spines in the lateral series of segments 2, 5 and 10. *Dracoderes orientalis* differs the most by missing lateroventral tubules on all three segments (SEM confirmation of the missing tubule on segment 10 is desirable though). Conversely, the two remaining species have lateroventral tubules on segments 2, 5 and 10, but can be differentiated by the lateral accessory spines on segment 5 that are present in *D. gallaicus* sp. nov. but absent in *D. abei*. Otherwise, the three species only differ in spine and segment dimensions, and in minor morphological details. The sensory spot distribution in *D. orientalis* still needs to be properly confirmed by SEM documentation, but detailed information for *D. abei* and *D. gallaicus* sp. nov. is provided in the present description, and the two species show striking resemblance, only deviating by the presence of paradorsal/perispinal sensory spots on segment 2 in *D. gallaicus* sp. nov, and this species' lack of ventrolateral sensory spots on segments 2 and 5.

The new, as yet undescribed species from the Okinawa area generally displays the same spine pattern as *D. abei*, even though the presence/absence of lateral accessory tubules on segment 8 and lateroventral tubules on segment 10 needs to be confirmed with SEM. However, the appearance of the dorsal spines differs strikingly from the three other species of the genus. Whereas the three described species all have thin, spinous and acicular spines in the dorsal series (see Figure 5A,C,G and 9A,D, but see also Figure 5.57 in Adrianov & Malakhov 1999), the dorsal spines in *Dracoderes* sp. 1 are much shorter and more plump, and with swollen bases (Figure 11A,B). To get a rough idea about the difference, the length/width ratio of the paradorsal spine of segment 7 in a specimen of *D. abei* is 20.2, whereas it is 8.8 in *Dracoderes* sp. 1. The special appearance of the dorsal spines were consistent in all 13 examined specimens, and would in itself make it easy to distinguish the species from all other known ones. In addition, the ventral part of the posterior segment margin of segment 2 forms a true and distinct pectinate fringe, whereas this margin does not show any particular differentiation in the other known species (compare, for example, Figure 5B and 11C).

Identity of Asian Dracoderes *specimens examined herein, and their conspecifity with* D. abei

When a species is redescribed, and the redescription is mainly based on novel, non-type material, it would always raise the question: how can we be sure that the examined specimens are conspecific with the redescribed species, and do not represent another, closely related one? In particular, the question is relevant if the emended diagnosis includes substantial differences and change of key characters in the original description. In the present case, the newly examined specimens of *D. abei* differ from the original description at several points, including number of placids in the neck (14 in the original description versus 9 in the redescription), the presence of lateral accessory tubules on segment 8 and lateroventral tubules on segment 10 (not mentioned in original description), and most prominently, the absence of a middorsal spine on segment 10 in males (original description reports a 28-μm long middorsal spine on segment 10 in the allotypic male). However, despite these differences, we find it justified to consider our specimens as conspecific with *D. abei*. Most importantly, we examined the male allotype of *D. abei* and found no trace of a middorsal spine on segment 10. Examination of specimens collected for the present study, only 16 km from the type locality of *D. abei*, revealed the same result. In this context, it is also noteworthy that the description of male morphology was based on examination of a single individual. If additional males had been included in the type series, the mistake would most probably never have occurred.

The interpretation of the neck placids was furthermore made much easier with information from SEM. Such information was not available for the original description. As for the tubules on segments 8 and 10, they could easily have been overlooked as well, since the original description was based on LM only. The lateral accessory tubules on segment 8 are extremely short, and often partly covered by the lateroventral spines when observed with LM. Furthermore, the underlying subcuticular structures associated with the spine attachments make it even harder to identify the attachment site of a tiny tubule. Also on segment 10, the subcuticular structures may have obscured the visualization of a tubule. The latter points are supported by LM observations made on the newly collected specimens, where tubules on segments 8 and 10 often were impossible to observe, even though their

presence could be confirmed by SEM examination of specimens from the same populations.

Based on this, we find it justified to consider the Korean and Japanese specimens collected for the present study as conspecific with *D. abei*. This is further supported by the congruent morphology of all examined specimens, including Korean ones, as well as those collected close to the type locality of *D. abei*. If more than one species of *Dracoderes* were present in the study area, we would expect to find at least some variation among the examined specimens.

Notes on selected morphological characters

Pharyngeal crown. In several groups of kinorhynchs, the pharyngeal bulb terminates frontally into a pharyngeal crown, formed by 10 tufts of heavily cuticularized microvilli (Kristensen & Higgins 1991; Neuhaus 1994). The presence of a pharyngeal cuticular crown was first reported by Zelinka (1928), who observed it with LM in serial sections of *Echinoderes dujardinii*, *Pycnophyes communis* and *Centroderes eisigii*. Subsequently, a pharyngeal crown has been documented with LM from *P. flaveolatus* (see Nyholm & Nyholm 1976), and with transmission electron microscopy (TEM) from *Kinorhynchus giganteus* (see Moritz & Storch 1972), *K. phyllotropis* (see Brown, 1989), *E. capitatus* (see Nebelsick 1993), *P. kielensis* and *P. dentatus* (see Neuhaus 1994). Interestingly, Neuhaus (1994) also confirms the absence of a pharyngeal crown in *Zelinkaderes floridensis* and questions its presence in *Cateria*.

Being a minute and internal structure, visualization of the pharyngeal crown usually requires examination of serial sections, and since such studies are rarely included in regular taxonomic or systematic studies, the available information about the structure is restricted to a few taxa only. By luck, some specimens examined in the present study showed abnormally protruded pharynxes which enabled us to confirm the presence of a pharyngeal crown in species of *Dracoderes* as well and, for the first time, to visualize the structure with SEM (Figure 3E and 8D).

In future studies, knowing that the presence of a pharyngeal crown in some instances can be confirmed with SEM may enable us to gather more information about the appearance of this structure across the kinorhynch taxa. In light of its presence in some but not all taxa, the pharyngeal crown may be a structure that could provide phylogenetically significant information; hence, in future systematic studies, we would urge morphologists to pay more attention to this somehow neglected structure.

Oral style and scalid distribution. The arrangement of inner oral styles corresponds well with all previous reports, except for the helioscalids that usually occur in the innermost ring (e.g. Brown 1989; Sørensen 2007; Sørensen et al. 2007). The outer oral styles in species of *Dracoderes* are unusual because they differ alternately in size. This alternation of sizes is only known from adults of two other genera, namely *Neocentrophyes* (see Higgins 1969) and *Paracentrophyes* (see Higgins 1983; Neuhaus 1995; Sørensen et al. 2010b). Sørensen et al. (2010a) furthermore reported a similar alternation in juvenile stages of *Antygomonas*, and suggested that alternating style sizes in adult specimens, as found among species of *Dracoderes*, *Neocentrophyes* and *Paracentrophyes*, could be paedomorphic character traits. A close relationship between species of *Neocentrophyes* and *Paracentrophyes* has been suggested previously and is supported by several other characters (Higgins 1983), but it remains uncertain if the alternating style sizes found among species of *Dracoderes* are homologous with the neocentrophyid condition, or whether they developed independently.

Except for the most posteriorly positioned scalids in introvert sections 1 and 6, the section-wise arrangement of introvert scalids in *D. abei* and *D. gallaicus* sp. nov. is identical with patterns found in several other species including *Kinorhynchus phyllotropis* (see Brown 1989), *Paracentrophyes praedictus* and *P. anurus* (see Neuhaus 1995; Sørensen et al. 2010b), *Tubulideres seminoli* (see Sørensen et al. 2007), all species of *Antygomonas* (see Bauer-Nebelsick 1996; Sørensen 2007; Sørensen et al. 2009), *Semnoderes armiger* (see Sørensen et al. 2009), *Wollunquaderes majkenae* (see Sørensen & Thormar 2010), and *Campyloderes* cf. *macquariae* (Sørensen, pers. obs.). The amount of reliable information about introvert morphology and scalid arrangements is still too limited and scattered across the taxa to make any conclusions, but some patterns start to become evident. For example, the species listed above indicate that there appears to be a broad range of taxa, represented by homalorhagid as well as cyclorhagid species that show almost identical scalid arrangements. Other groupings of taxa that deviate from this pattern are species of *Zelinkaderes* and *Triodontoderes* that have reduced the number of scalid rings (Higgins 1990; Bauer-Nebelsick 1995; Sørensen et al. 2007; Sørensen & Rho 2009), and species of *Cephalorhyncha* and *Echinoderes* where additional scalids often appear in the most posterior rings (Nebelsick 1993; Sørensen 2008; Sørensen & Pardos 2008; Sørensen pers. obs. on several undescribed species of *Echinoderes*). Further data from additional species are obviously required to draw any conclusions, but the increasing amount of

information clearly shows that the introvert morphology could provide new clues about evolutionary pathways within Kinorhyncha.

The nephridial pores. The excretory system of kinorhynchs always consists of a pair of protonephridia located in segments 8 and 9, with nephridial pores opening laterally on segment 9 (Kristensen & Hay-Schmidt 1989; Kristensen & Higgins 1991). In species of Echinoderidae, the protonephridial openings form two fairly conspicuous sieve plates, and due to their distinct appearance in LM as well as SEM, they are often reported in systematic and taxonomic studies. However, in non-echinoderid species there are no sieve plates and the nephridial pores are much more inconspicuous. Hence, reports on nephridial outlets from these taxa are very sporadic. They include only a few species of *Pycnophyes* (Neuhaus 1988, 1994; Higgins & Kristensen 1991), *Kinorhynchus phyllotropis* (see Brown 1985) and *Zelinkaderes floridensis* (see Neuhaus 1994), and in all cases the documentation has been based on TEM examinations of sectioned specimens. The epicuticular appearance of a nephridial pore in a non-echinoderid has not been documented previously; hence, it came as a surprise that this feebly visible structure actually could be visualized with SEM (Figure 6G and 10B), and prompted a closer examination of SEM photos of other species from previous studies of the authors. It appears that similar pores are present in sublateral or lateral accessory positions in at least *Antygomonas incomitata*, *Campyloderes* cf. *macquariae*, *Condyloderes megastigma*, *Pycnophyes barentsi*, *Semnoderes armiger* and *Tubulideres seminoli*. The minute sublateral tubules on segment 9 in *Wollunquaderes majkenae* (see Figure 6D,E in Sørensen & Thormar 2010) might also be connected to the nephridial system. Visualization with SEM of nephridial pores in non-echinoderid species requires very clean specimens and detailed examinations, but the present results indicate that it is possible to locate the pores, and future morphological studies should be done with this in mind.

Conclusions

Based on the emended diagnosis of *Dracoderes abei*, we can conclude that the species has a much wider distribution than known previously, and has been recorded so close to the type locality of *D. orientalis* that their distributional ranges most likely overlap. The finding of a new species of *Dracoderes* in Spain, Western Europe, demonstrates that *Dracoderes* is not a strictly Asian genus, and implies that additional species of the genus may show up in other places of the world. The latter is also confirmed by the recording of the yet undescribed species, *Dracoderes* sp. 1, from the Okinawa region.

In the future, the new information revealed by SEM may contribute to our understanding of kinorhynch phylogeny and evolution. Even though species of *Dracoderes* may resemble species of Echinoderidae superficially (e.g. due to the lack of midterminal spine in adult specimens), several characters such as the scalid arrangement and appearance of the nephridial pore show more resemblance with other, non-echinoderid cyclorhagids and even homalorhagids. The alternating sizes of the outer oral styles is only shared with species of two homalorhagid genera, *Neocentrophyes* and *Paracentrophyes*, and may indicate an even closer affinity between *Dracoderes* and Homalorhagida than previously thought. This leads *Dracoderes* to become a key taxon in studies of kinorhynch evolution and stresses the importance of the taxon in future, phylogenetic analyses.

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Chapter IV

New Kinorhyncha from Florida coastal waters

Nuevos kinorrincos de las aguas costeras de Florida

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En este trabajo se describen cuatro nuevas especies del filo kinorrincos procedentes de la costa de Fort Pierce, Florida, USA en el Atlántico Occidental. Estas nuevas especies son: Antygomon*as gwnae* n. sp., *Echinoderes riceae* n. sp., *Echinoderes adrianovi* n. sp. y *Pycnophyes norenburgi* n. sp. Todas ellas se obtuvieron en la misma localidad de muestreo, denominada "estación 20 millas". Las muestras se procesaron para obtener datos granulométricos estandarizados, obteniéndose un diámetro de partículas medio estimado de 250 µm. También se discuten los caracteres diagnósticos y la morfología general de las nuevas especies en profundidad, así como la diversidad y distribución del filo Kinorrincos en el área.

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ORIGINAL ARTICLE

New Kinorhyncha from Florida coastal waters

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Abstract Four new species of Kinorhynchs are described from the West Atlantic coast off Fort Pierce, Florida, USA. They are the following: *Antygomonas gwenae* n. sp., *Echinoderes riceae* n. sp., *Echinoderes adrianovi* n. sp. and *Pycnophyes norenburgi* n. sp. All species were collected at the same locality called "20 miles station." Samples were processed for standard granulometric data, yielding an estimated average particle diameter of 250 μm. The diagnostic characters and the general morphology of the new species are discussed in depth as well as the diversity and distribution of Kinorhyncha in the area.

Keywords Kinorhyncha · Meiofauna · Florida · *Echinoderes* · *Antygomonas* · *Pycnophyes*

Introduction

The kinorhynch fauna reported from the East coast of the USA includes currently 14 species. The first three kinorhynch species recorded and described in the area were *Echinoderes remanei* Blake 1930; *Pycnophyes frequens* Blake 1930; and *Kinorhynchus mainensis* Blake 1930. These three species were found to form a common assemblage from Maine to Massachusetts (Wieser 1960; Higgins 1964a, 1965). Two additional species, *Campyloderes* sp. reported as *Campyloderes macquariae* Johnston 1938 by Higgins (1980) and posteriorly assigned to *Campyloderes* cf. *vanhoeffeni* by Neuhaus and Sørensen (2013); and *Centroderes spinosus* Reinhard 1881 recognized as a new species of *Centroderes* by Neuhaus, Pardos, Sørensen and Higgins (in preparation), were found later by Higgins (1982) in the same area. The latter species was not identified correctly, and his recording represents a yet undescribed species. Blake's three species are replaced by three similar species in the area stretching from Beaufort, North Carolina to Florida. They include *Pycnophyes beaufortensis* Higgins 1964; *Kinorhynchus langi* Higgins 1964; and *Echinoderes bookhouti* Higgins 1964 (see Higgins 1964b). The kinorhynch fauna of Bermuda reported by Higgins (1982) includes *Echinoderes bispinosus* Higgins 1982; *Echinoderes bermudensis* Higgins 1982; *Kinorhynchus fimbriatus* Higgins 1982; and a new species of *Centroderes*. All of the above species were collected subtidally, but in 1977, two new cyclorhagid kinorhynchs were found in intertidal habitats. *Echinoderes sublicarum* Higgins 1977 was found in bryozoan colonies on pilings in a tidal stream in the North River Inlet, South Carolina, and *Echinoderes coulli* Higgins 1977 and was found in the mud of this same intertidal creek area. This latter species was the first kinorhynch published with a complete series of juvenile developmental stages (see Higgins 1977).

The knowledge of the marine fauna in Atlantic Florida waters has been improved along many years by the research activities developed through the Smithsonian Marine Station at Fort Pierce, FL. It became well known among researchers that these waters constitute a kind of hot spot for meiobenthic communities. Accordingly, study sites were established in 5-mile increments seaward from Fort Pierce, Florida, between 1975 and 1995 by R. P Higgins, a

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research effort that focused mainly on the phylum Kinorhyncha. Several new species appeared but only *Zelinkaderes floridensis* Higgins 1990 was described formally. *Z. floridensis* is a cyclorhagid kinorhynch, which represented a new family, the Zelinkaderidae (see Higgins 1990). This species occurs primarily at depths around 140 m in the muddy sand found 20 miles offshore. More recently, M. V. Sørensen collected at different localities off Fort Pierce and described four new species including a new genus: *Echinoderes spinifurca* Sørensen et al. 2005, *Zelinkaderes brightae* Sørensen et al. 2007, *Antygomonas paulae* Sørensen 2007 and *Tubulideres seminoli* Sørensen et al. 2007 (see Sørensen et al. 2007).

The purpose of this paper is to describe the remaining species from Bob Higgins' old samplings from the deepest and richest of the stations hereafter referred to as "20 miles station" combining his results with new records from recent samplings.

Materials and methods

Sediment samples were taken in August 1993 from one single station: the "20 miles site," 27°30′N, 79°56′W at 140-m depth (Fig. 1) from sandy mud using a Higgins anchor dredge (Higgins and Thiel 1988) from the R/V Sunburst (Smithsonian Marine Station at Fort Pierce). The anchor dredge was designed to sample only the upper few centimeters of sediment, thereby eliminating the need for processing large volumes of otherwise uninhabited sediment. Additional material was collected in August 2011, at the same station from the same R/V and using the same dredge.

Sediment samples for granulometric studies were wet-sieved using fresh water to remove salt through a series of geological sieves with 0.5-phi (φ) intervals. Phi (φ) = $-\log_2$ of mesh size in mm (1,000, 500, 250, 125 and 63 µm mesh). A mechanical shaker was used for a period of 15 min. The material retained on each sieve was placed in disposable aluminum weighing pans and dried in an oven at 80 °C for 24 h. After drying, each fraction of the material was weighted. The sediment weight fractions (calculated in percent of the total sample) (Fig. 2a) were transformed into a cumulative frequency series and then plotted as a cumulative frequency curve (Fig. 2b). In the latter, the medium particle diameter, i.e., the φ value corresponding to the 50 % point of the cumulative scale (Md φ or φ 50), is estimated. The "quartile deviation" (QD) expresses the number of phi (φ) units lying between the upper and lower quartile diameters Q1 and Q3 (Fig. 2b). Thus, sediment with a small spread between the quartiles, i.e., a small QD φ, is regarded as being "well sorted" (Higgins and Thiel 1988).

In the samples from 1993, kinorhynchs were sorted under a stereomicroscope, fixed in 10 % formalin and, after 24 h, transferred to 20 % Carosafe ® (Carolina Biological Supply, Co.). Selected specimens were later transferred to 2 % glycerin in 70 % ethanol, which was allowed to evaporate over a period of about 5 days, thereby leaving the specimens in glycerin only. From the glycerin, each specimen was transferred to a drop of Hoyer's 150 mounting medium (Higgins and Thiel 1988) on the base coverslip of an H–S slide mount (Higgins and Thiel 1988; Shirayama et al. 1993). A 12-mm-diameter, circle cover glass was placed on the mounting medium and manipulated to orient the specimen.

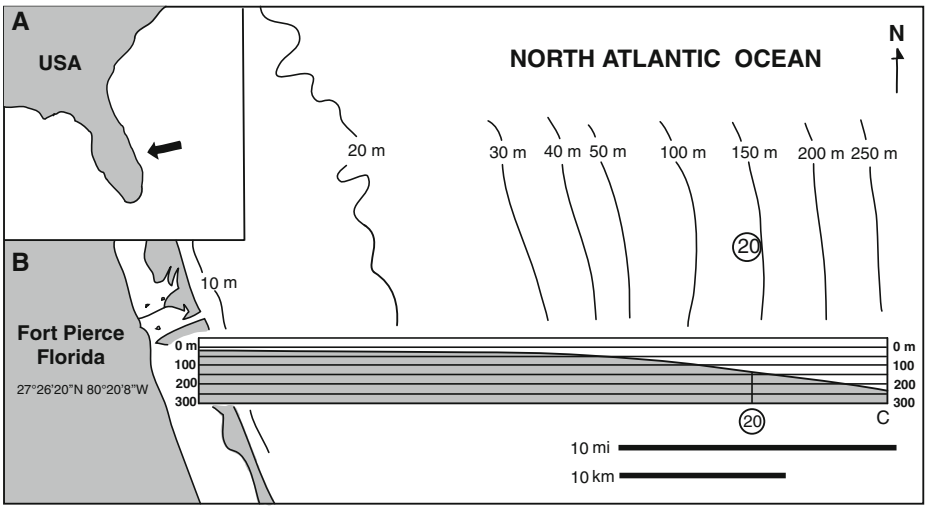

Fig. 1 Map showing **a** Florida (inset), western North Atlantic Ocean. **b** Locality of the study off Fort Pierce Station corresponding to miles off shore indicated by numbers in oval. **c** Transect showing depths from 0 to 300 m with *vertical line* corresponding to the 20 miles station

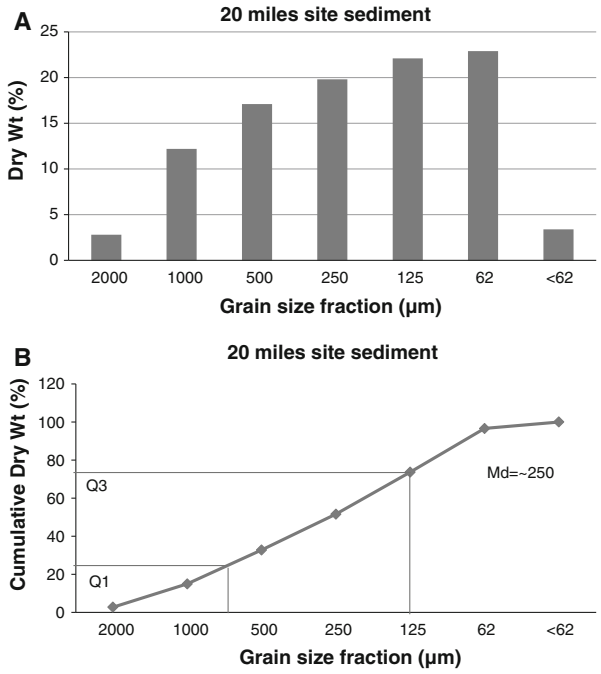

Fig. 2 Grain size fractions plotted: **a** against dry weight (%) **b** against cumulative dry weight (%). *Md* medium particle diameter. *Q1,3* quartile diameters

Specimens collected in 2011 were fixed in 10 % formalin. For light microscopy (LM) selected specimens were later dehydrated through a graded series of ethanol and transferred to glycerin prior to mounting in Fluoromount G®. All specimens were examined and photographed using an Olympus BX51 microscope with differential interference contrast optics equipped with an Olympus Colorview DP70 camera. Measurements were made using Olympus Cell A software (Olympus, Europe).

Not all species described yielded specimens suitable for scanning electron microscopy (SEM) studies. The available specimens for SEM were dehydrated through a graded series of ethanol and critical point dried. The dried specimens were mounted on aluminum stubs, sputter coated with gold and examined with a JEOL JSM-6335F field emission scanning electron microscope.

Terminology for head, neck and trunk morphology follows Neuhaus and Higgins (2002), Sørensen and Pardos (2008) for cyclorhagids and Sánchez et al. (2011) for homalorhagids. The number and distribution of introvert appendages has been studied both by rings and sectors using polar diagrams following Sørensen and Pardos (2008). The terminology used to describe the different kinds of scalids follows both Brown (1989) and Higgins (1990), recently revised and unified by Neuhaus (2012).

Results

Granulometry

Results of the sediment analyses (Fig. 2) show that a high percentage of the dry weight of the sediment is represented by grain size fractions ranging from 500 to 62 µm, with 62 µm occurring as the most frequent grain size constituting 23 % of the sediment, followed by 125 µm with 22 % and 250 µm with 17 %. Grain fractions from 250 to 62 µm constitute around 64 % of the total sediment. Cumulative dry weight graphic shows a higher slope in between 250 and 62 µm as well, showing a mean particle size around 250 µm. The grain size fractions less represented are the smallest <62 µm and the biggest 2,000 µm with a 2.8 and 3.4 %, respectively. However, the amount of the fraction smaller than 62 µm is not totally reliable due to the use of 63-µm mesh to take the samples. That means that part of the finest fraction could have been washed out during the collection in the periphery of the dredge.

Taxonomic account

Antygomonas gwenae n. sp. (Figs. 3, 4; Tables 1, 2)

Order Cyclorhagida (Zelinka 1896) Higgins 1964
Family Antygomonidae Adrianov and Malakhov 1994
Genus *Antygomonas* Nebelsick 1990

Diagnosis

Antygomonas with a very sclerotized cuticle. Anterior edge of the first trunk segment with a conspicuous notch in middorsal and midventral positions extending half of the segment length. Trunk segments 2–10 with distinct tergosternal junctions. Middorsal spines from segments 1–4 and 10 are flexible and thin, while those from segments 5–9 and 11 are conical, robust and stout. Segment 2 with a pair of lateroventral cuspidate spines located close to a pair of much smaller, flexible and hairy acicular spines. Segments 3 through 9 with lateroventral acicular spines; segments 5, 8 and 9 additionally with pair of lateroventral, lateral accessory or ventrolateral cuspidate spines. Acicular spines become very robust and stout from segment 5 in advance especially in segments 8 and 9. Cuspidate spines in lateroventral position on segments 5 and 9; cuspidate spines on segment 8 in lateral accessory position. Segment 10 with laterodorsal acicular spines flexible in males and stout and straight in females. Lateral terminal spines much shorter than lateral terminal accessory spines. Females with minute midventral conical projection of segment 9. Males differing from females by the presence of crenulated posterior half of middorsal and subdorsal spines of segment 10

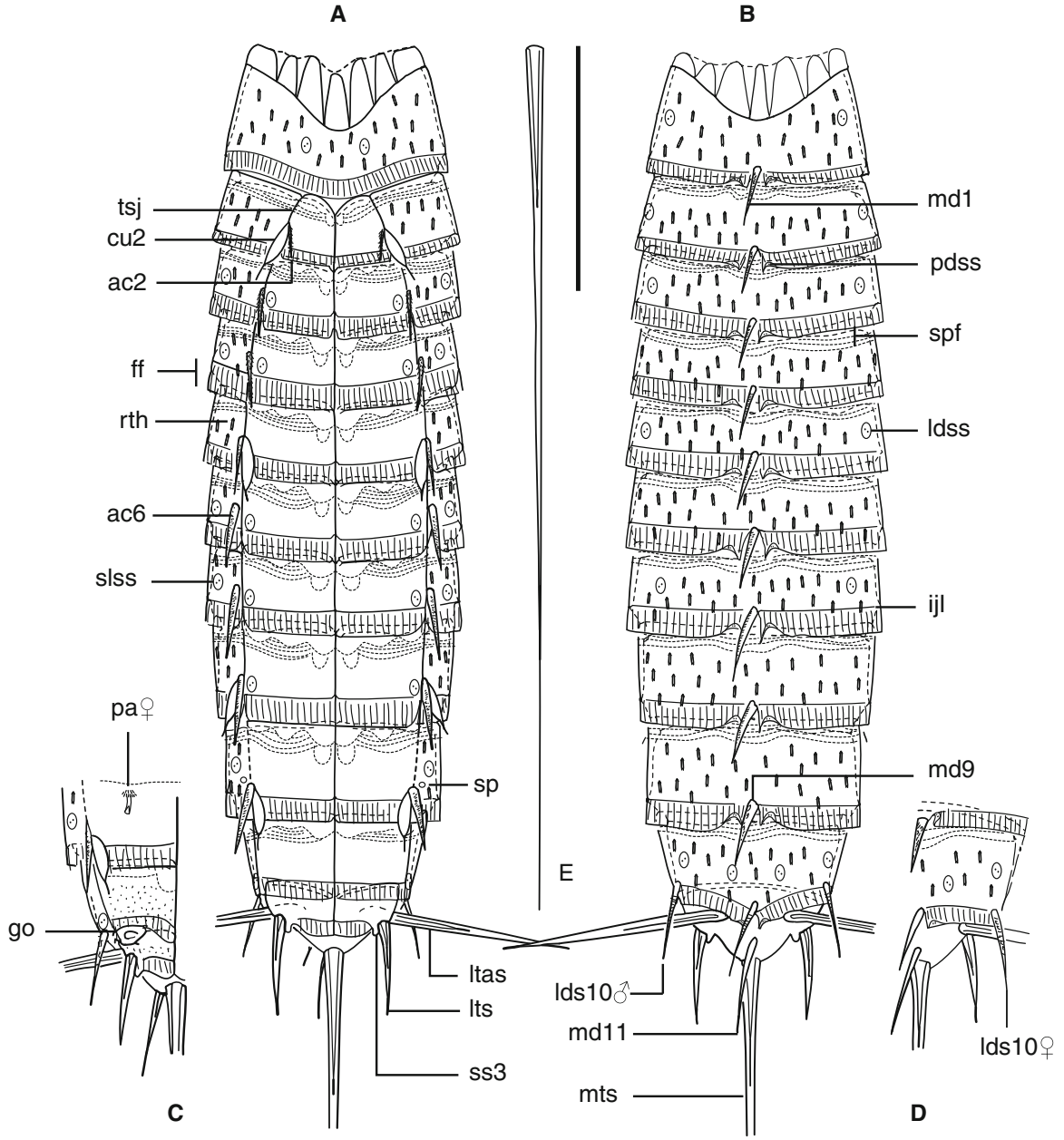

Fig. 3 *Antygomonas gwenae* n. sp. *line art* illustration. **a** Male ventral view. **b** Male dorsal view. **c** Female ventral detail of segments 10–11. **d** Female dorsal detail of segments 10–11. **e** Midterminal spine full length. *ac* acicular spine, *cu* cuspidate spine, *ff* free flap, *go* gonopore, *ijl* intersegmentary joint line, *lds* laterodorsal spine, *ldss* laterodorsal sensory spot, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *md* middorsal spine, *mts* midterminal spine, *pa* papillae, *pdss* paradorsal sensory spot, *rth* roof tile hairs, *ss3* sensory spot type 3, *slss* sublateral sensory spot, *sp* sieve plate, *spf* secondary pectinate fringe, *tsj* tergosternal junctions. Digits refer to the segment number. *Scale bar* 100 μm

and the lack of a pair of strongly sclerotized gonopores on segment 10.

Etymology

This species is named in honor Gwen L. Higgins, Bob Higgins' wife of through 50 years.

Type material

Holotype: adult male collected in August 1993 at the 20 miles station, Fort Pierce, off the Floridian West coast (Fig. 1) from sandy mud, mounted in Hoyer's medium. Allotype: adult female, same collecting data as holotype, mounted in Hoyer's medium. Paratype: adult female, same

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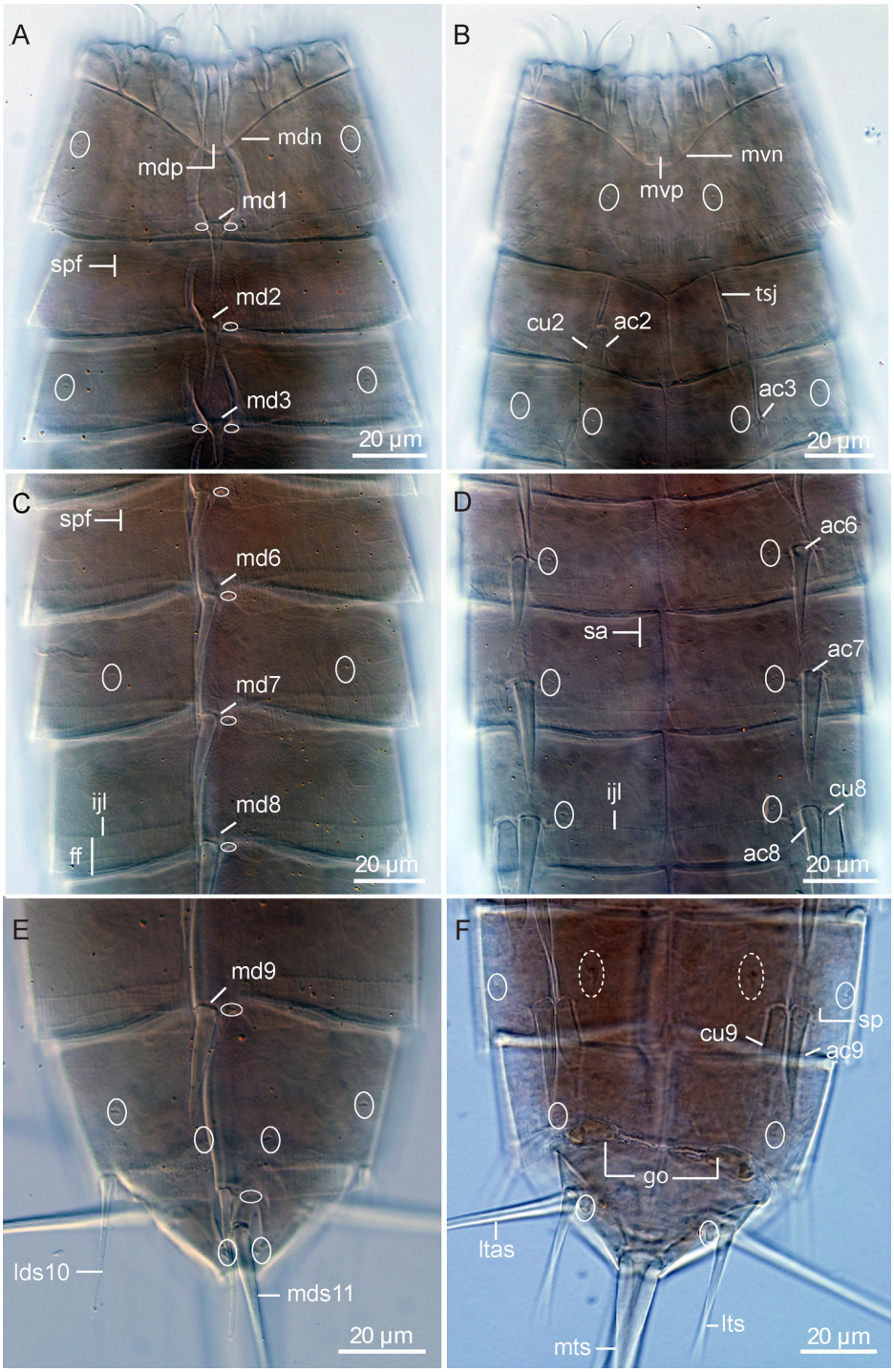

Fig. 4 Light micrographs showing details in neck and trunk morphology of *Antygomonas gwenae* n. sp. holotypic male USNM 1196402 (**a**–**e**) and allotypic female USNM 1196402 (**f**). **a** Neck and segments 1–3, dorsal view. **b** Neck and segments 1–3 ventral view. **c** Segments 6–8, dorsal view. **d** Segments 6–8 ventral view**. e** Segments 9–11, dorsal view. **f** Segments 9–11 ventral view. *ac* acicular spine, *cu* cuspidate spine, *ff* free flap, *go* gonopore, *ijl* intersegmentary joint line, *lds* laterodorsal spine, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *md* middorsal spine, *mdn* middorsal notch, *mdp* middorsal placid, *mts* midterminal spine, *mvn* midventral notch, *mvp* midventral placid, *sa* smooth area, *sp* sieve plate, *spf* secondary pectinate fringe, *tsj* tergosternal junctions. Digits after abbreviations refer to segment number. *White circles* indicate sensory spots (*solid line*) and papillae in females (*dotted line*)

Table 1 Measurements of adult *Antygomonas gwenae* n. sp. (in μm)

	Holotype male	Paratype male	Allotype female		Holotype male	Paratype male	Allotype female
TL	356	372	264	MD5	24	23	25
SW	40	62	62	MD6	24	26	27
SW/TL (%)	11.2	16.7	23.5	MD7	30	22	28
MSW-8	76.8	78.8	78.0	MD8	28	23	28
MSW/TL (%)	21.6	21.1	29.5	MD9	28	25	30
MTS	352	200	288	MD10	32	25	35
MTS/TL (%)	98.8	53.2	109.0	LDS10	33	35	28
S1	42	38	40	MD11	40	31	49
S2	31	28	26	LVS2 (cu)	24	22	18
S3	32	31	29	LVS2 (ac)	13	11	13
S4	34	34	32	LVS3	22	18	20
S5	37	35	35	LVS4	24	21	22
S6	38	38	35	VLS5 (cu)	28	22	24
S7	38	42	36	LVS5 (ac)	23	24	26
S8	41	42	40	LVS6	28	26	28
S9	42	44	42	LVS7	31	30	30
S10	44	43	40	LVS8 (ac)	32	30	30
S11	30	32	42	LAS8 (cu)	25	28	28
MD1	23	22	24	VLS9 (cu)	28	32	31
MD2	21	23	23	LVS9 (ac)	30	23	24
MD3	23	23	24	LTS11	40	41	41
MD4	24	25	25	LTAS11	89	80	80

Numbers, where inserted, indicates segment number

ac Acicular spine, *cu* cuspidate spine, *las* lateral accessory spine, *lds* laterodorsal spine, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *md* middorsal spine, *msw* maximum sternal width, *mts* midterminal spine, *sw* standard width, *s1–s11* segment lengths of trunk segments 1–11, *tl* trunk length, *vls* ventrolateral spine. Holotypic male in boldface

Table 2 Summary of nature and location of sensory spots, spines and papillae arranged by series in *Antygomonas gwenae* n. sp

Segments	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	ac	ss		ss						ss
2	ac	ss			ss			cu, ac		
3	ac	ss		ss		ss		ac	ss	
4	ac	ss				ss		ac	ss	
5	ac	ss		ss				ac	cu	
6	ac	ss				ss		ac	ss	
7	ac	ss		ss		ss		ac	ss	
8	ac	ss					cu	ac	ss	
9	ac	ss				ss		ac	cu	pa♀
10	ac	ss	ss	ac, ss				ss		
11	ac, mts	ss, ss					ltas	lts	ss	

ac Acicular spine, *cu* cuspidate spine, *la* lateral accessory, *ld* laterodorsal, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lv* lateroventral, *md* middorsal, *ml* midlateral, *mts* midterminal spine, *pa* papilla, *pd* paradorsal, *sd* subdorsal, *sl* sublateral, *ss* sensory spot, *vl* ventrolateral, *vm* ventromedial, ♀ female condition of sexually dimorphic character

collecting data as holotype and allotype, mounted in Hoyer's medium. Holotype and allotype were deposited at the National Museum of Natural History (Smithsonian Institution) under accession numbers USNM 1196402 and USNM 1196403, respectively. The single paratype is stored in the Meiofauna Laboratory collection at the Facultad de Biología, Universidad Complutense de Madrid under accession number K15/31.

Description

Adult specimens consisting of a head, a neck and eleven trunk segments (Figs. 3, 4) Measurements and dimensions are given in Table 1. A summary of cuticular characters (spines, tubules, gland outlets and sensory spots) locations is given in Table 2.

Mouth cone and introvert armature could not be examined in detail in any of the prepared specimens.

Neck with 16 placids of unequal shape, width and length. Midventral and middorsal placids are triangular and elongated measuring 6 µm wide at their bases and 22 µm long, narrower than all others (Figs. 3a, b, 4a, b). Laterally adjacent placids in the dorsal side still triangular being 14 µm wide, longer mesially than laterally but overall slightly longer than middorsal and midventral placids. Ventral placids narrower measuring 8 µm in width and 20 µm in length. Remaining placids more rectangular, nearly equal in size, 10 µm wide and 12 to 16 µm long. The cuticle between adjacent placids is more flexible, folding inwards and giving the false appearance of interstitial placids. No trichoscalid plates could be observed.

Segment 1, 42 µm long at lateral margins, 26 µm long middorsally and midventrally, forming an anterior broad notch (Figs. 3a, b, 4a, b). The cuticle in this and the following segments seems to be thicker and stronger than in other *Antygomonas* species. Middorsal spine short, hairy and flexible originating near the posterior edge of the segment in a notched margin and flanked by paradorsal sensory spots in this and the following segments. Paired sensory spots are furthermore present in laterodorsal and ventromedial positions (Figs 3a, b, 4a, b). Sensory spots in this and the following segments are rounded with short papillae and two pores, appearing very distinct in light microscopy; paradorsal sensory spots with a semicircular outline (Fig. 3b). It was not possible to identify the sensory spot type. Hence, more detailed examination through scanning electron microscopy is required to provide an exact map of these structures. Cuticular hairs are scattered over the segment. The hairs seem to have a roof tile or elongated leaf-like appearance with a perforation site, and are scattered around the segment. Wide striated free flap delimited anteriorly by a conspicuous ij-line in this and the following segments (Figs. 3a, b,4c). Pectinate fringe not apparent through LM.

Segment 2, 31 µm long at lateral margin of tergal plate, narrowing to 25 µm long at tergosternal junction. Sternal plates with arch-shaped anterior margins, each being ca. 16 µm wide anteriorly and 23 µm wide posteriorly (Figs. 3a, 4b). A pair of lateroventral cuspidate spines present, each with an associated hairy, thin and flexible acicular spine (Figs. 3a, 4b). Middorsal spine similar to that of previous segment. Paired sensory spots in paradorsal and midlateral positions. The anterior part of the tergal plate has at least four secondary pectinate fringes arranged into parallel rows on this and the following segments (Figs. 3b, 4a). All of them consist of a wavy line of minute, cuticular, spine-like scales; the last row is enlarged middorsally forming a conspicuous fringe behind the middorsal spine of each segment. Anterior to the secondary fringes a narrow smooth area without any scales occurs; this area is usually covered by the free posterior edge of the preceding segment (Fig. 4a–c). Secondary pectinate fringes on the sternal plates made by similar scales arranged into a wavy lines making out a conspicuous smooth area (Figs. 3a, 4d). Cuticular hairs with the same structure as on preceding segment but present on tergal plate only. Free flap striated and without a marked pectinate fringe as on previous segment. Middorsal and lateroventral spines on this and the following segments are associated with a conspicuous notch of the free flap (Figs. 3b, 4a, c, d).

Segment 3. Sternal plates slightly trapezoid, narrowest at anterior margins. (Figs. 3a, 4b). Flexible and hairy lateroventral spines present. Middorsal spine similar to that of previous segment. Paired sensory spots in paradorsal, laterodorsal, sublateral and ventrolateral positions. Cuticular hairs, fringes and free flap as on preceding segment.

Segment 4. Sternal plates nearly squarish. Middorsal spine slightly longer and more robust than spine on previous segments. Lateroventral acicular spines with the same appearance as those from segment 3. Paired sensory spots in paradorsal, sublateral and ventrolateral positions. Cuticular hairs, fringes and free flap as on previous segments.

Segment 5 with a middorsal spine slightly longer and stronger than the one of the previous segment (Fig. 4c). A pair of ventrolateral cuspidate spines longer than those of segment 2 closely associated with a pair of lateroventral and stout acicular spines both present with the same length (Fig. 3a). Both the middorsal and the lateral accessory acicular spines with a thick-walled cuticle around a central cavity. Paired sensory spots in paradorsal and laterodorsal positions. Cuticular hairs, fringes and free flap as on previous segment.

Segment 6 with a stout middorsal spine with the same length as the one in previous segment. A pair of wide and stout lateroventral acicular spines is present (Figs. 3a, 4d). Both the middorsal and lateroventral spines with a thick-walled cuticle around a central cavity, in this and the following segments. Paired sensory spots situated on paradorsal, sublateral and ventrolateral positions. Cuticular hairs, fringes and free flap as on previous segments.

Segment 7 with a stout middorsal spine (Figs. 3b, 4c). A pair of robust lateroventral spines is present (Figs. 3a, 4d). Sternal plates similar to those of segment 6, slightly narrowing at posterior edges. Sensory spots located in

paradorsal, laterodorsal, sublateral and ventrolateral positions. Cuticular hairs, fringes and free flap as in previous segments.

Segment 8 with a very robust middorsal spine (Fig. 3b). Lateroventral acicular spines also very stout (Fig. 3a). Both middorsal and lateroventral acicular spines with the same appearance as described in previous segments. A pair of lateral accessory cuspidate spines is present. Paired sensory spots situated in paradorsal and ventrolateral positions. Cuticular hairs, fringes and free flap as in previous segments.

Segment 9 with a stout middorsal spine (Figs. 3b, 4e). Paired ventrolateral cuspidate spines and conspicuously robust lateroventral acicular spines present (Figs. 3a, 4f). Paired sensory spots in paradorsal and sublateral positions. Female with a pair of ventromedial conical protuberances or papillae (modified gland cell outlets) (Figs. 3c, 4f). Small oval sieve plate near lateroventral tergal margin, long axis about 6 µm (Figs. 3a, 4f). Cuticular hairs, fringes and free flap as on previous segments.

Segment 10 showing either a thin and crenulated middorsal spine in males or a stout middorsal spine in females (Figs. 3b, d, 4e, f). Additionally, a pair of laterodorsal spines crenulated in males and robust in females with the same length of the middorsal spine is present. Paired sensory spots in paradorsal, subdorsal laterodorsal and lateroventral positions. The posterior margin of the tergal plate free flap has deep notches around the middorsal and laterodorsal spines. Female's free flap of the sternal plates with two ventrolateral rounded notches around the gonopores of segment 11; this free flap is straight in males (Figs. 3a, c, 4f). Cuticular hairs and pectinate fringes as on previous segments.

Segment 11 triangular, tapering posteriorly. Tergal plate slightly longer than sternal plates without tergal extensions. Segment appendages consist of a middorsal spine, a midterminal spine nearly the same length as the trunk and paired lateral terminal and lateral terminal accessory spines (Figs. 3a, b, e, 4e, f). The lateral accessory spines are twice as long as the lateral terminal spines. Paired modified sensory spots (type 3) are present at the base of the middorsal, midterminal and lateral terminal spines (Figs. 3a, b, 4e, f). These sensory spots consist of an area with densely set papillae, with a long and slender tubule protruding from the center. Female gonopores are easily recognized in LM as two strongly cuticularized areas at the intersegmental junction between segments 10 and 11 (Figs. 3c, 4f). Cuticular leaf-like hairs are lacking in this segment, instead the surface is covered by small scale-like hairs without perforation sites. A wide and striated free flap is present only in the sternal plates, lacking in tergal ones. Secondary fringes could not be examined in detail.

Remarks

The new species has sixteen placids, a first trunk segment being ring-shaped showing middorsal and midventral anterior notches, both presence of acicular and cuspidate spines, cuticular structures such as hairs and secondary fringes, and characteristic notches in the posterior margins of the trunk segments. All those characters are only shared by species of *Antygomonas.* However, there are some other important features of the new species that do not fit that well within this genus such as the high sclerotization of the trunk cuticle, the depth of the notches on segment 1 (around 50 % of the segment length), very short and robust middorsal and lateroventral spines, middorsal and laterodorsal spines of segment 10, being crenulated only in males, and the presence of distinct tergosternal junctions in segments 2–11. Based on the last difference solely, one would have to reject the assignment of the new species to the genus *Antygomonas* as originally described by Nebelsick (1990). However, and regarding this feature, Sørensen (2007) reported the presence of hardly recognizable tergosternal divisions in *A. paulae* and re-examined specimens of *Antygomonas oreas* Bauer-Nebelsick 1996, indicating that all segments from 2 to 11 consist of one tergal and two sternal plates. Subsequently, Sørensen et al. (2009) confirmed this trait also for *Antygomonas incomitata.* Thus, with the emended diagnosis based on these examinations, we can consider the genus *Antygomonas* to have segments 2–11 weakly divided into one tergal and two sternal plates; therefore, the new species can be still considered as belonging to *Antygomonas*.

Within the suborder Conchorhagae, the genera *Semnoderes* and *Sphenoderes* share several notable characters with the new species such as the composition of the placids, combining different lengths and widths; segment 1 with more or less developed incisions or notches that could function similar to the clam-shell-like closing apparatus described for *Sphenoderes* and *Semnoderes* (see Higgins 1969; Sørensen et al. 2009; Sørensen et al. 2010; Zelinka 1928); the presence of short and stout spines (only shared with *Sphenoderes* species) and the presence of the conical papillae located in the center of each sternal plate of segment 9. However, there are some noteworthy variations in the closing apparatus: *Semnoderes* has a segment one composed of two lateral halves separated by deep but not complete incisions and a neck with narrow middorsal and midventral placids to fit into these incisions (Sørensen et al. 2009). *Sphenoderes* in turn has a first segment divided into single tergal and sternal plates and a pair of lateral plates (Higgins 1969), showing broader incisions with also broader middorsal and midventral placids. In case of *Sphenoderes poseidon* Sørensen and Thormar 2010, the first segment is clearly not divided, but the broader

incisions and placids are still present. The new species has a first segment not divided but showing conspicuous although not very deep anterior notches. Furthermore, in the neck, the midventral and middorsal placids are conspicuously narrower than the remaining ones. These differences exclude the new species to be assigned as either *Semnoderes* or *Sphenoderes*.

The doubtful position of the new species in between *Antygomonas*, *Semnoderes* and *Sphenoderes* is not unexpected knowing all the similarities shared by those genera previously reported by Sørensen et al. (2009), (2010).

Surprisingly, the new species shares other characters with species of the genus *Centroderes* such as the sexual dimorphism exhibited by the laterodorsal and middorsal spines of segment 10, being crenulated in males; this feature is not shared by any species of *Antygomonas* nor *Semnoderes* and *Sphenoderes.* However, other genera such as *Wollunquaderes*, *Tubulideres* (Sørensen et al. 2007); *Triodontoderes* (Sørensen and Rho 2009); and *Zelinkaderes* (Higgins 1990; Bauer-Nebelsick 1995; Sørensen et al. 2007) show the same dimorphism. The stout and robust middorsal and laterodorsal acicular spines of *A. gwenae* n. sp. resemble those showed in *Centroderes,* although species of *Semnoderes* and especially *Sphenoderes* (*S. poseidon* Sørensen et al. 2010) also share this character. Future phylogenetic analysis would hopefully clarify whether all these traits are relevant for taxonomy and phylogenetic relationships.

The combination of the traits displayed by the new species makes its generic assignment problematic and somehow resembles the situation described for *Wollunquaderes majkenae* (see Sørensen and Thormar 2010), which has characters pointing toward Semnoderidae and Centroderidae. We tentatively assign the new species to the genus *Antygomonas* despite all the differences listed above until phylogenetic analysis confirm or reject it. Another alternative could be to erect a new cyclorhagid intermediate genus to accommodate this single species, based on all the differences showed with the genus *Antygomonas* and the similarities shared with *Sphenoderes* and *Semnoderes*. The authors find it inadequate without a phylogenetic background. Thus, as stated above, the new species fits better within *Antygomonas* than in other alternative genera and therefore is here assigned provisionally to this genus.

Currently, the genus *Antygomonas* comprises three species: *Antygomonas incomitata* Nebelsick 1990; *A. oreas;* and *A. paulae*. The main differences between these species are basically their spine formula, or more precisely the position of the cuspidate spines on segments 6, 8 and 9 (see Nebelsick 1990; Bauer-Nebelsick 1996; Sørensen 2007). *Antygomonas gwenae* n. sp. is distinguished from *A. incomitata* by the lack of ventrolateral cuspidate spines on segment 9 and lateroventral cuspidate spines on segment 8. They also differ in length of the LTS and LTAS being equal in *A. incomitata* while different in *A. gwenae* n. sp. which shows much shorter LTS. *A. oreas* and *A. gwenae* n. sp. can be discriminated by the position of the cuspidate spines on segment 9, which is lateroventral in *A. gwenae* n. sp. and lateral accessory in *A. oreas.* Also the TL of *A. oreas* is around 50 µm shorter than *A. gwenae* n. sp. *A. paulae* and *A gwenae* n. sp. have exactly the same spine formula, but they can be easily discriminated by the body dimensions with *A. paulae* being 100 µm longer than *A. gwenae* n. sp. and by the overall appearance of the middorsal and lateroventral spines stout and short in *A. gwenae* n. sp. and long and flexible in *A. paulae*. *A. gwenae* n. sp. can furthermore be differentiated from *A. paulae* by the sensory spot pattern showing additional sensory spots in laterodorsal position on segments 3 and 5 and in sublateral positions on segments 3, 6, 7 and 9. The lack of ventral notches of segments 1, 3, 4 and 6–8 occurring in A. paulae and lacking in A. gwenae and the different length of the LTAS and LTS equal in *A. paulae* are as well important features to distinguish both species (see Sørensen 2007).

Echinoderes adrianovi n. sp. (Figs. 5, 6; Tables 3, 4)

Order Cyclorhagida (Zelinka 1896) Higgins 1964
Family Echinoderidae Zelinka 1894
Genus *Echinoderes* Claparède 1863

Diagnosis

Echinoderes with long middorsal spines on segments 4–8 increasing in length from segments 4–7, middorsal spine of segment 8 shorter than the one on segment 7; long subdorsal and ventrolateral tubules on segment 2; lateroventral tubules on segment 5; lateroventral spines on segments 6–9; lateral accessory tubule on segment 8; lateral accessory spines on segment 11 in females; lateral terminal spines on segment 11.

Etymology

This species is named in honor of Dr. Andrey V. Adrianov, Vice Director, Institute of Marine Biology, Far East Branch, Russian Academy of Science, Vladivostok, Russia, a student, mutual friend and colleague of R.P. Higgins.

Type material

Holotype: adult female collected on August 1993 at the 20 miles site, Fort Pierce, off the Floridian West coast (Fig. 1) 27°30′N, 79′56′W, 140 m depth from sandy mud, mounted in Hoyer's medium, deposited at National Museum of

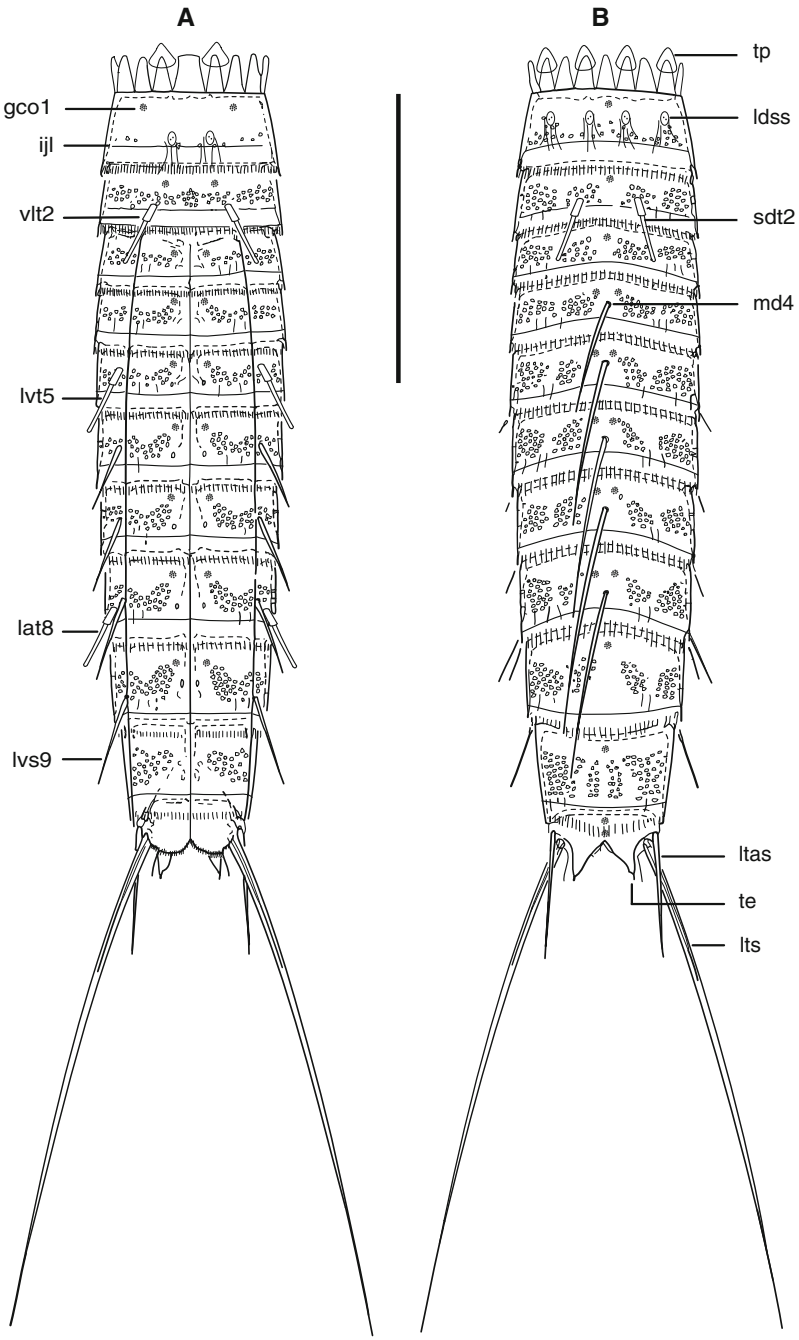

Fig. 5 *Echinoderes adrianovi* n. sp. *line art* illustration. **a** Ventral view. **b** Dorsal view. *ijl* intersegmentary joint line, *lat* lateral accessory tubule, *ldss* laterodorsal sensory spot, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *lvt* lateroventral tubule, *md* middorsal spine, *gco1* glandular cell outlet type 1, *sdt* subdorsal tubule, *te* tergal extension, *tp* trichoscalid plate, *vlt* ventrolateral tubule. Digits refer to the segment number. *Scale bar* 100 μm

Natural History (Smithsonian Institution) under accession number USNM 1196401.

Description

Holotypic female with head, neck and 11 trunk segments (Figs. 5a, b, 6a). See Table 3 for measurements and dimensions. A summary of location of cuticular characters (spines, tubules, gland outlets and sensory spots) is given in Table 4. No specimens were available for SEM examination; thus, it was not possible to identify unambiguously all minor cuticular structures such as sensory spots and gland cell outlets. These structures are reported when apparent through LM; however, the lack of mention in the description of some segments should not be interpreted as a confirmation of their absence.

The head consists of a retractable mouth cone and an introvert. Inner and outer armature could not be examined in detail.

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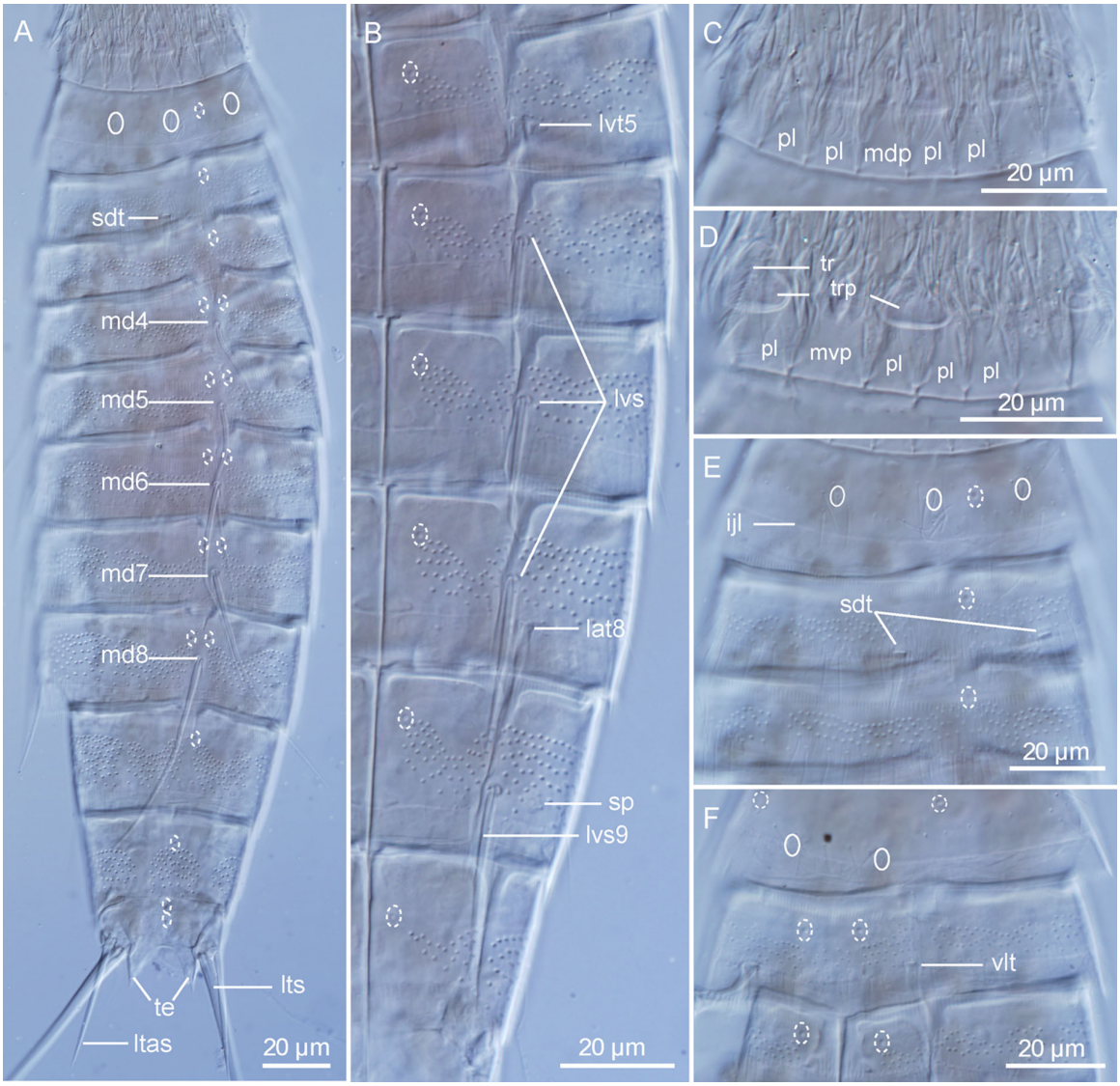

Fig. 6 Light micrographs showing details in neck and trunk morphology of *Echinoderes adrianovi* n. sp. holotypic female, USNM 1196401. **a** Dorsal view. **b** Segments 5–10 left side, ventral view. **c** Neck, dorsal view. **d** Neck lateroventral view. **e** Segments 1–3, dorsal view. **f** Segments 1–3, ventral view. *ijl* intersegmentary joint line, *lat* lateral accessory tubule, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *lvt* lateroventral tubule, *md* middorsal spine, *mdp* middorsal placid, *mvp* midventral placid, *pl* placid, *sdt* subdorsal tubule, *sp* sieve plate, *te* tergal extension, *tp* trichoscalid plate, *tr* trichoscalid, *vlt* ventrolateral tubule. *White circles* indicate glandular cell outlets type 1 (*dotted line*) and sensory spots (*solid line*). Digits after abbreviations refer to segment number

The neck consists of 16 placids all measuring 12 µm in length; midventral placid 10 µm wide at the base; and 8 µm distally where it expands laterally at its margins (Figs. 5a, 6c); remaining placids measure 6 µm at their bases and 3 µm distally. All placids articulate with the first trunk segment. Six trichoscalid plates present, 2 ventral on placids 1 and 16 and 4 dorsal on placids 6, 8, 10 and 12 (Figs. 5a, b, 6c, d). Ventral trichoscalid plates are triangular with rounded corners and wider than dorsal ones which have a triangular outline (Figs. 6c, d).

Trunk

The trunk has 11 segments, with segments 1 and 2 consisting of a closed cuticular ring, and segments 3–11 with one tergal and two sternal plates (Fig. 5a).

Table 3 Measurements (in µm) of adult *Echinoderes adrianovi* n. sp. holotype

Character	Holotype	Character	Holotype
TL	262	MD4	38
SW	42	MD5	56
SW/TL (%)	16	MD6	64
MSW-8	42	MD7	78
MSW/TL (%)	16	MD8	64
S1	28	VLT2	24
S2	28	DLT2	24
S3	24	LVS5	24
S4	24	LVS6	24
S5	26	LVS7	28
S6	29	LVS8	30
S7	30	LAT8	27
S8	34	LVS9	32
S9	36	LTS11	174
S10	40	LTAS11	44
S11	28		

Numbers, where inserted, indicates segment number

dlt Dorsolateral tubule, *lat* lateral accessory tubule, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *md* middorsal spine, *msw* maximum sternal width, *sw* standard width, *s1–s11* segment lengths of trunk segments 1–11, *tl* trunk length, *vlt* ventrolateral tubule

Table 4 Summary of nature and location of sensory spots, glandular cell outlets, spines and tubules arranged by series in *Echinoderes adrianovi* n. sp

Segments	MD	PD	SD	LD	SL	LA	LV	VL	VM
1	gco1		ss	ss			gco1		ss
2	gco1		tu					tu	gco1
3	gco1								gco1
4	ac	gco1							gco1
5	ac	gco1					tu		gco1
6	ac	gco1					ac		gco1
7	ac	gco1					ac		gco1
8	ac	gco1				tu	ac		gco1
9	gco1				sp		ac		gco1
10	gco1								gco1
11	gco1, gco1					ltas	lts		

ac Acicular spine, *gco1* glandular cell outlet type 1, *la* lateral accessory, *ld* laterodorsal, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lv* lateroventral, *md* middorsal, *pd* paradorsal, *sd* subdorsal, *sl* sublateral, *sp* sieve plate, *ss* sensory spot, *tu* tubule, *vl* ventrolateral, *vm* ventromedial

Glandular cell outlets type 1 (pore fields) are situated either at the anterior part of the segments on areas surrounded by perforation sites (ventrally) or located always anterior to the perforation site area (dorsally). Ventral pore fields are located in a lateroventral position on segment 1 and paraventrally on segments 2–10 (Figs. 5a, 6b). Dorsal pore fields are unpaired middorsally on segments 1–3, 9–10 and paradorsal pairs on segments 4–8 (Figs. 5b, 6a). Segment 11 has two unpaired middorsal pore fields above each other (Fig. 6a).

Segment 1 consists of one complete cuticular ring. Three pairs of type 1 sensory spots present in subdorsal, laterodorsal and ventromedial positions (Figs. 5a, b, 6a, f). Cuticular hair pattern distributed above the ij-line (Figs. 5a, b). Wide free flap terminates into a fine pectinate fringe.

Segment 2 consists of one complete cuticular ring. Two pairs of tubules, subdorsal and ventrolateral, are present (Figs. 5a, b, 6e, f). Cuticular perforation sites prominent and distinctly patterned forming a continuous band around the segment interrupted in laterodorsal and paraventral areas; the longest hairs are situated in the last row. Free flap and pectinated fringe as on preceding segment.

Segment 3. This and the following segments consist of one tergal and two sternal plates. Spines or tubules are not present. Cuticular hair pattern very distinct showing a dense band around the segment interrupted in laterodorsal and paraventral areas; the last row shows the longest hairs. Free flap and pectinated fringe as on preceding segment.

Segment 4 with a middorsal spine situated midway from anterior margin of tergal plate (Figs. 5b, 6a). Cuticular hair pattern as on segment 3 but also interrupted in middorsal position. Other features similar to previous segment.

Segment 5 with a middorsal spine and one pair of lateroventral tubules (Figs. 5a, 6a, b), otherwise similar to segment 4.

Segments 6 and 7 with a middorsal spine and one pair of lateroventral spines (Figs. 5a, b, 6a, b), otherwise similar to segment 4.

Segment 8 with a middorsal spine and one pair of lateroventral spines plus lateral accessory tubules (Figs. 5a, b, 6a, b), otherwise similar to segment 4.

Segment 9 with a pair of lateroventral spines, no middorsal spine is present. Paired rounded and small sieve plates are found in a sublateral position (Fig. 6b). Otherwise similar to preceding segments.

Segment 10 with a distinctive rounded perforation site patch middorsally separated from the lateral cluster (Fig. 5b, 6a); sternal pattern more uniformly clustered than on preceding segment. Oval muscle scars near posterior midline of each sternal plate. Other characters similar to previous segments.

Segment 11 smooth; tergal plate with prominent and pointed tergal extensions (Figs. 5b, 6a); sternal plates with rounded posterior margins, with minute pectinate fringe and single prominent long hair extending posteriorly, in line with tergal extensions; lateral terminal spines 66.4 % of trunk length; lateral terminal accessory spines 25 % of the lateral terminal spine length.

Remarks

Currently forty-one species of *Echinoderes* share the same middorsal spine pattern (segments 4–8). Of these, twenty-one species show ventrolateral tubules on segment 2, lateroventral tubules on segment 5 and lateroventral spines on segments 6–9. Only one species out of these twenty-one, *Echinoderes kanni* Thormar and Sørensen 2010, described from the Solomon Islands, share the pattern of spines/tubules with *E. adrianovi* n. sp. Despite the coincidence of spine/tubule formula and body proportions, both species can be differentiated by the length of these spines and tubules: *E. kanni* shows all middorsal spines increasing progressively in length, while *E. adrianovi* n. sp. shows the middorsal spine of segment 8 shorter than the one on previous segment being equal in length with the one on segment 6. The middorsal spines of segments 4–7 in *E. adrianovi* n. sp. are around 10 µm longer than in *E. kanni,* except for the middorsal spine on segment 8 which is 20 µm shorter. The length of the ventrolateral and dorsolateral tubules of segment 2 show big differences measuring twice as much in *E. adrianovi* n. sp.; tubules of segments 5 and 8 are also longer in *E. adrianovi* n. sp. but not as long as those on segment 2. Lateroventral spines show differences as well being slightly longer in *E. adrianovi* n. sp. except for the lateroventral spine on segment 9 much longer in *E. kanni.* Lateral terminal and lateral terminal accessory spines around 15 µm longer in *E. adrianovi* n. sp.

Another species that strongly resembles *E. adrianovi* n. sp. is *Echinoderes parrai* GaOrdóñez et al. 2008, described from the North coast of Spain (GaOrdóñez et al. 2008). This is the only other species of the genus *Echinoderes* combining 5 middorsal spines, dorsal tubules on segment 2 and lateral accessory tubules on segment 8. However, it lacks ventrolateral tubules on segment 2, it has much shorter LTS and LTAS and shows a bulbous appearance of the anteriormost segments; therefore, it is very easily discriminated from *E. adrianovi* n. sp.

Two other species of the same genus, *Echinoderes spinifurca* and *Echinoderes horni* Higgins 1983, are reported from the Atlantic coast of Fort Pierce, Florida, USA (Sørensen et al. 2005). Both have been found closer to shore, from the coarser sediments of the 5 miles station. *E. horni* is otherwise known only from its type locality, Carrie Bow Cay, Belize. *E. adrianovi* n. sp. is easily differentiated from *E. spinifurca* because of the very long tergal extensions and the lack of tubules laterodorsally on segment 2 and lateral accessory on segment 8 of the latter species, and from *E. horni* because this latter lacks middorsal spines.

Echinoderes riceae n. sp. (Figs. 7, 8, 9, 10; Tables 5, 6)

Diagnosis

Echinoderes with middorsal spines on segments 4, 6 and 8 gradually increasing in length posteriorly; ventrolateral tubules on segment 2; lateroventral tubules on segment 5; lateroventral spines on segments 7, 8 9; lateral accessory tubules on segment 8.

Etymology

This species is named in honor of Dr. Mary E. Rice, Emeritus Research Zoologist at the Smithsonian Marine Station at Fort Pierce, Florida, for a whole life dedicated to the marine research.

Type material

Holotype: adult female collected on August 1993 at the 20 miles site, Fort Pierce, off the Floridian West coast (Fig. 1) 27°30′N, 79′56′W, 140 m depth from sandy mud, mounted in Hoyer's medium, deposited at the National Museum of Natural History (Smithsonian Institution) under accession number USNM 1196386. Allotype: adult male, same collecting data as holotype, mounted in Hoyer's medium, deposited at National Museum of Natural History (Smithsonian Institution) under accession number USNM 1196396. Paratypes, 9 males and 9 females same collecting data as holotype, mounted in Hoyer's medium. Thirteen paratypes were deposited at the National Museum of Natural History under accession numbers USNM 1196387-95 and USNM 1196397-99. Remaining five paratypes are preserved in the Meiofauna Laboratory collection at the Facultad de Biología, Universidad Complutense de Madrid under accession numbers K15/31-36.

Additional material

Additional material was collected in August 2011 at the 20 miles site, Fort Pierce, off the Floridian West coast 27°30,84′N 79° 54,86′W; 152 m depth from fine mud. Ten specimens were studied in LM mounted in Fluoromount G®, and 5 were prepared and examined with SEM. This material is deposited in the Meiofauna Laboratory collection at the Facultad de Biología, Universidad Complutense de Madrid under accession numbers K15/1-10.

Description

Adult specimens consist of a head, a neck and eleven trunk segments (Figs. 7a, b, 9a, b). Measurements and dimensions are given in Table 5. A summary for location of sensory spots, spines, tubules and glandular cell outlets is provided in Table 6.

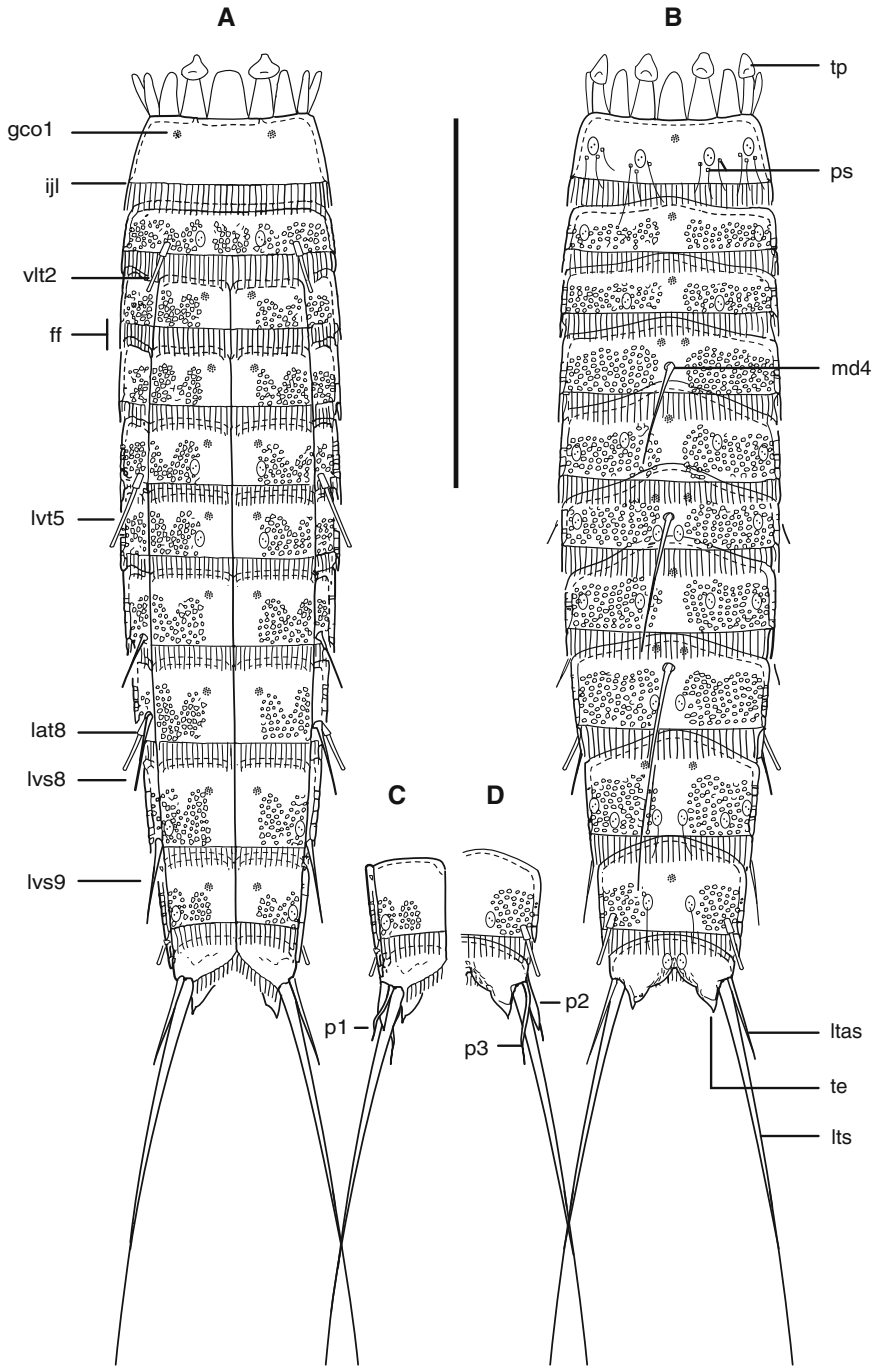

Fig. 7 *Echinoderes riceae* n. sp. line art illustration. **a** Ventral view. **b** Dorsal view. **c** Male detail segment 10–11 ventral view. **d** Male detail segments 10–11, ventral view. *ff* free flap, *ijl* intersegmentary joint line, *lat* lateral accessory tubule, *ldss* laterodorsal sensory spot, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *lvt* lateroventral tubule, *md* middorsal spine, *p1-3* penile spines, *gco1* glandular cell outlet type 1, *ps* perforation sites, *te* tergal extension, *tp* trichoscalid plate, *vlt* ventrolateral tubule. Digits refer to the segment number. *Scale bar* 100 µm

The head consists of a retractable mouth cone and an introvert (Fig. 8). The mouth cone has nine, long and pointed outer oral styles divided into two segments. The bases have a conspicuous long fringe composed of at least five fringe tips flanked by a pair of spikes (Fig. 10a); the inner armature of the mouth cone could not be examined in detail. The introvert has seven rings of spinoscalids and one additional ring of trichoscalids (Fig. 8). Ring 01 has ten primary spinoscalids consisting of a short basal sheath, equipped with two rows of long fringes and a long end piece. Ring 02 is formed by 10 laterally compressed spinoscalids, all formed by a basal part covered with a long (more than half of the length of the scalid) and smooth sheath that terminates into a short fringe with wide fringe tips (Fig. 10b). Ring 03 with 20 spinoscalids all with conspicuous sheaths around their bases and a

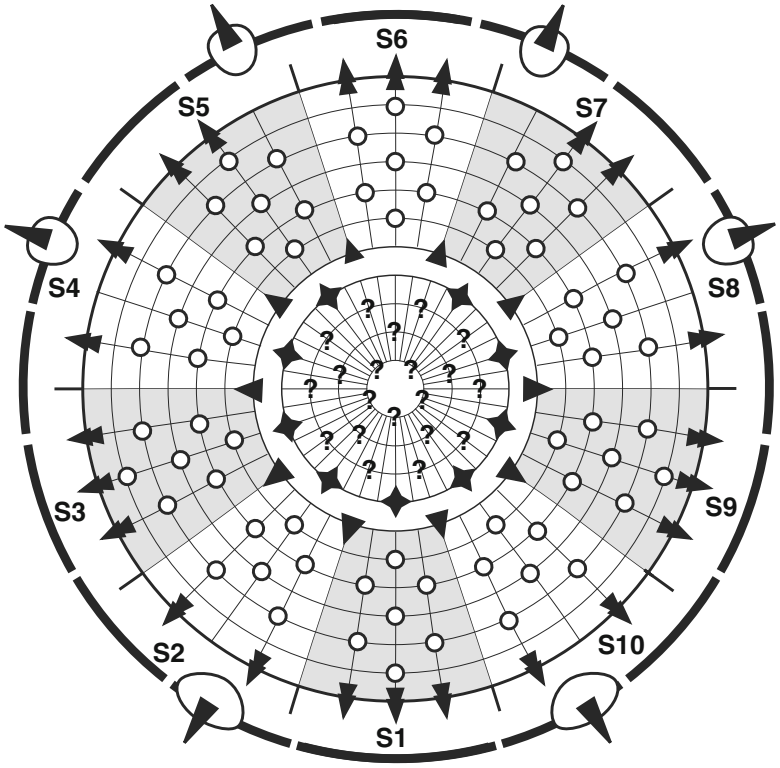

Fig. 8 Diagram of mouth cone, introvert and placids showing distribution of oral styles and scalids in *Echinoderes riceae* n. sp. "*Double diamonds*" are marked in the table with *double lines,* and quincunxes are marked with *dotted lines*. *ls* leaf-like scalid, *oos* outer oral style, *psp* primary spinoscalid, *S1-10* sectors, *sps* spinoscalid, *tr* trichoscalid

Scalid and style arrangement

Ring/Sector	1	2	3	4	5	6	7	8	9	10	Total
00 oos ✦	1	1	1	1	1	0	1	1	1	1	9
01 psp ▼	1	1	1	1	1	1	1	1	1	1	10
02 sps ○	1	1	1	1	1	1	1	1	1	1	10
03 sps ○	2	2	2	2	2	2	2	2	2	2	20
04 sps ○	1	1	1	1	1	1	1	1	1	1	10
05 sps ○	2	2	2	2	2	2	2	2	2	2	20
06 sps ○	1	0	1	0	1	1	1	0	1	0	6
07 ls	3	2	3	2	2	3	2	2	3	2	24
08 tr	0	1	0	1	1	0	1	1	0	1	6
Total scalids	10	9	10	9	10	10	10	9	10	9	115

distal fringe. These sheaths have rounded edges with a characteristic flexible spine in the middle (Fig. 10b). Rings 04 and 05 consist of 10 and 20 spinoscalids, respectively (Fig. 8); all resemble those of ring 03 but without the conspicuous sheaths. Ring 06 is formed by 6 spinoscalids, shorter than those in preceding rings and also showing shorter sheaths. Ring 07 has 20 leaf-like scalids with a wide and hairy base from where several flexible elongations arise (Fig. 10b). Six trichoscalids are present on their respective trichoscalid plates (Figs. 8, 9a, b, 10b). The introvert can also be described as divided into ten sectors defined by radii drawn through primary spinoscalids, so each sector is delimited by two consecutive primary spinoscalids (Fig. 8). The midventral sector is numbered as 1, followed clockwise by sectors 2–10. All sectors, except the middorsal sector 6, contain one oral style in the mouth cone. Even sectors contain 8 spinoscalids (other than primary ones) except for sector 6 with 10 spinoscalids. Uneven sectors contain 10 spinoscalids except for sectors 5 and 7, which also contain a trichoscalid that prevents the presence of one of the leaf-like scalids. The arrangement of the spinoscalids shows double diamonds in uneven sectors and sector 6 and quincunxes in the remaining even sectors formed by spinoscalids of rows 03–05 (Fig. 8). Trichoscalid plates with trichoscalids are situated on sectors 2, 4, 5, 7, 8 and 10. See Fig. 8 for a complete summary of oral styles, scalids and placid locations.

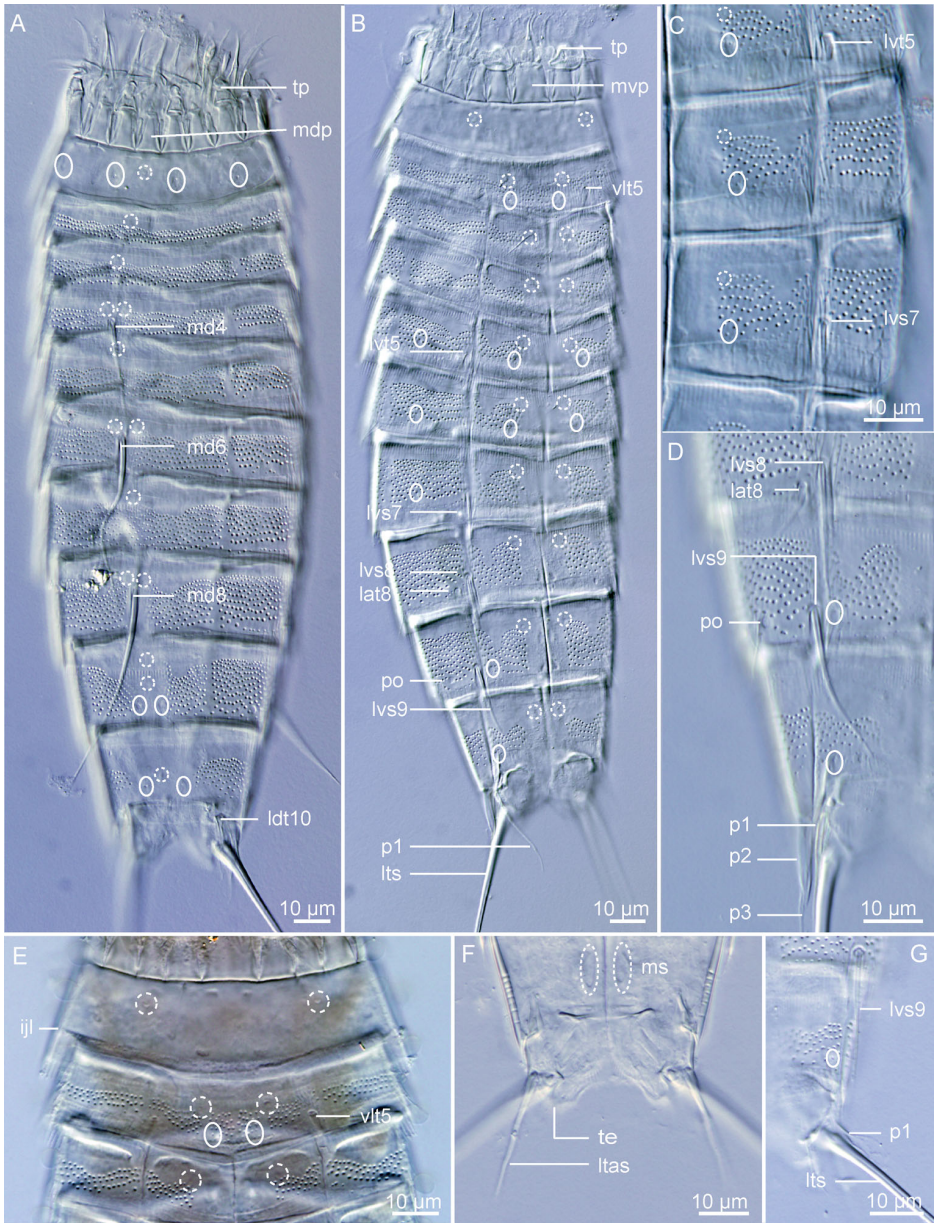

Fig. 9 Light micrographs showing details in neck and trunk morphology of *Echinoderes riceae* n. sp. Non-types. **a** Male dorsal view. **b** Male ventral view. **c** Detail of segments 5–7, ventral view, left side. **d** Male, detail of segments 8–11, ventral view, right side. **e** Detail segments 1–3, ventral view. **f** Female details segments 10–11, ventral view, focus is on the ltas. **g** Male detail of segments 10–11 ventral view, left side. *ijl* intersegmentary joint line, *lat* lateral accessory tubule, *ldt* laterodorsal tubule, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *lvt* lateroventral tubule, *md* middorsal spine, *mdp* middorsal placid, *ms* muscular scar, *mvp* midventral placid, *po* protonephridial opening, *p1-3* penile spines, *te* tergal extension, *tp* trichoscalid plate, *vlt* ventrolateral tubule. *White circles* indicate glandular cell outlets type 1 and (*dotted line*) and sensory spots (*solid line*). Digits after abbreviations refer to segment number

Neck with 16 placids, 13 µm long, conventionally numbered clockwise from the midventral one at sector 1. Midventral placid 10 µm wide at base, distal margin of placid nearly the same width. Lateral placids, 6 µm wide at base, 3 µm wide at distal margin. All placids articulate with the first trunk segment. Trichoscalid plates appear on dorsal placids 6, 8, 10, 12 and on ventral placids 2 and 16. Dorsal trichoscalid plates are rounded and small (6 µm), while ventral plates are larger and triangular, with enlarged bases (9 µm).

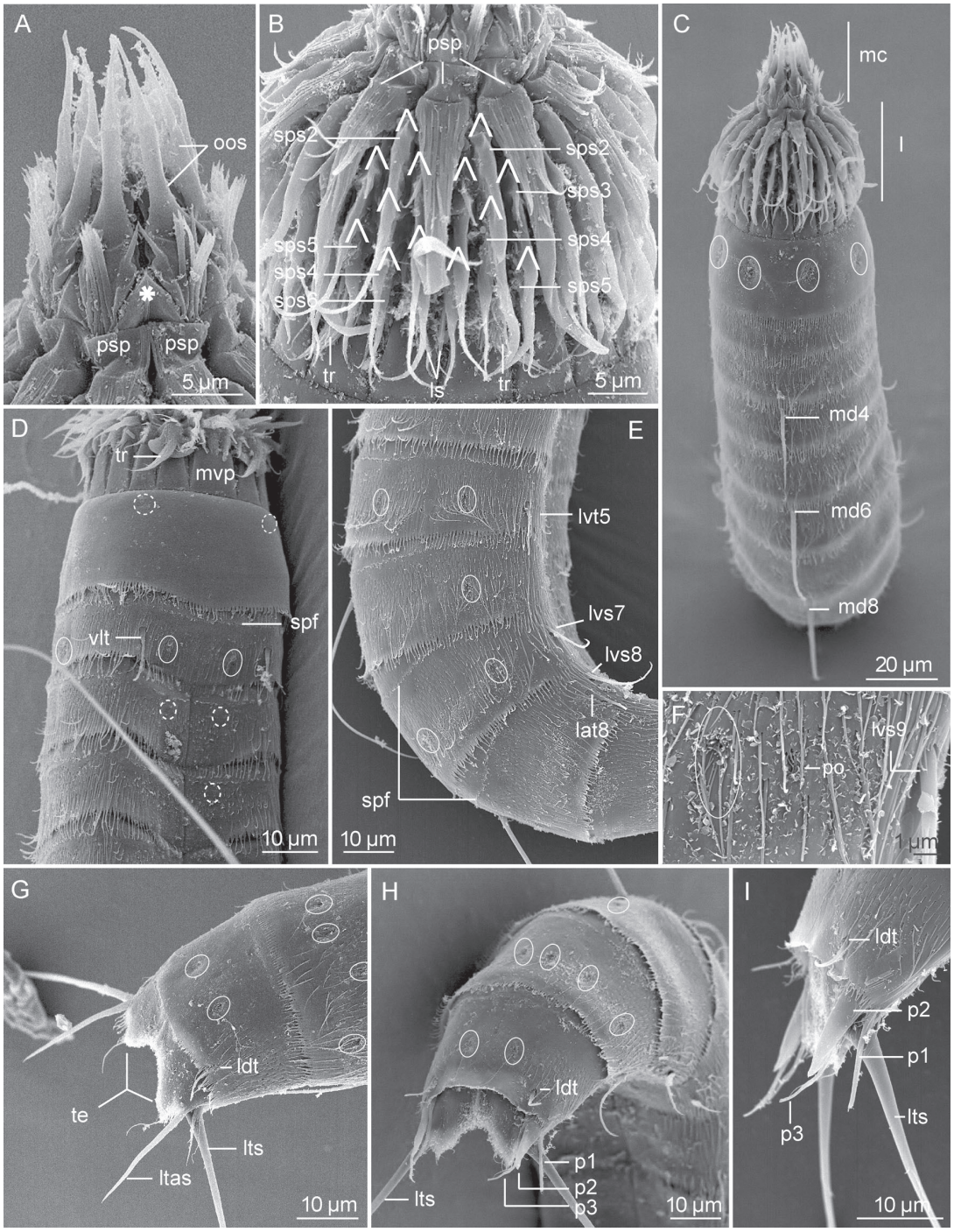

Fig. 10 Scanning electron micrographs showing trunk morphology and cuticular details in *Echinoderes riceae* n. sp. **a** Detail of mouth cone, *asterisk marks* middorsal position. **b** Detail introvert, sectors 4–5. **c** Introvert, neck and segments 1–8, dorsal view. **d** Detail of neck and trunk segments 1–4, ventral view. **e**. Detail of trunk segments 5–8, lateral view. **f** Detail of protonephridial opening on segment 9, lateral view. **g**. Female detail of trunk segments 9–11, dorsal view. **h**. Male detail of trunk segments 8–11, laterodorsal view. **i**. Male detail of penile spines on segment 11, lateral view. *I* introvert, *lat* lateral accessory tubule, *ldt* laterodorsal tubule, *ls* leaf-like scalid, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *mc* mouth cone, *md* middorsal spine, *mvp* midventral placid, *po* protonephridial opening, *oos* outer oral style, *p1-3* penile spines, *psp* primary spinoscalid, *sps* spinoscalid, *spf* secondary pectinate fringe, *te* tergal extension, *tr* trichoscalid, *vlt* ventrolateral tubule. *White circles* indicate glandular cell outlets type 1 (*dotted line*) and sensory spots (*solid line*). Digits after abbreviations refer to segment number or introvert ring number. *Lambda* symbols (Λ) mark attachment points of scalids

Trunk

Type 1 glandular cell outlets (pore fields) are ventrally situated at the anterior part of the segments, or dorsally located always anterior to the perforation sites (Figs. 7a, b, 9a, b). Ventral pore fields are located in a ventrolateral position on segment 1, paraventral position on segments 2–10. Dorsal pore fields are unpaired middorsally on segments 1, 2, 3, 5, 7, 10 and paired in a paradorsal position on segments 4, 6, 8, 9.

Secondary pectinate fringe present in the anterior margin of segments 2–11 as a continuous belt of short fringe tips (Figs. 10d, e).

Segment 1 consists of one complete cuticular ring. Pairs of subdorsal and laterodorsal sensory spots flanked by two or three long cuticular hairs emerging from rounded perforation sites present. Those are the only cuticular hairs noted both dorsally or ventrally in the segment (Figs. 7a, b, 9a, e, 10c, d) giving the cuticle a smooth appearance. Sensory spots on this segment are big, rounded with numerous papillae and two pores. Posterior margin with a short and well-developed pectinate fringe.

Segment 2 consists in one complete cuticular ring. A pair of ventrolateral tubules present. Each tubule consists of a short and smooth basal part, and a longer distal part with two small longitudinal, wing-like lateral projections (Figs. 7a, 9e, 10d). Paired sensory spots present in ventromedial and laterodorsal positions. Sensory spots in this and the following segments are smaller and more oval showing less papillae. These papillae increase in length at

Table 5 Measurements (in µm) for adults of *Echinoderes riceae,* n. sp

Character	Type		*n*		Range		Average		SD	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
TL	240	222	9	10	206–240	196–248	226	224	10.9	16.0
SW (%)	28	32	8	8	26–38	29–37	30	33	4.3	3.4
SW/TL (%)	11.7	14.4	8	8	12–17	12–18	13	15	1.9	1.8
MSW-10	44	42	7	8	40–48	38–45	44	41	2.4	2.3
MSW/TL	18.3	18.9	7	8	18–22	17–21	19	18	1.2	1.4
S1	23	24	8	8	23–26	23–28	24	25	1.3	1.6
S2	23	22	8	8	21–24	20–26	23	23	1.3	1.8
S3	22	24	8	8	21–27	22–26	24	24	2.1	1.4
S4	24	24	8	8	23–28	22–28	25	24	2.3	1.3
S5	25	26	8	8	25–30	24–32	27	28	2.2	1.4
S6	28	26	8	8	26–33	26–34	28	30	2.7	2.3
S7	32	32	8	8	27–34	28–34	31	32	2.6	2.3
S8	31	34	8	8	30–34	30–34	33	30	1.5	1.6
S9	32	34	8	8	31–34	30–33	32	21	1.2	1.4
S10	28	32	8	8	27–30	29–22	28	32	1.5	1.4
S11	18	22	8	8	18–20	19–38	19	33	0.7	0.9
MD4	24	24	9	9	17–26	20–34	23	26	3.4	5.1
MD6	50	48	9	9	32–56	38	46	49	7.6	5.2
MD8	64	60	7	10	60–70	57–44	63	62	3.6	2.9
VLT2	11	l6	9	10	10–16	10–15	12	15	2.4	2.1
LVS5	16	16	9	10	12–21	10	16	15	2.9	1.9
LVS7	20	20	9	10	18–28	20–26	21	21	3.0	1.4
LVS8	24	24	9	10	21–30	22–26	24	24	3.0	1.4
LAT8	16	19	7	10	16–21	14–32	18	20	2.0	2.5
LVS9	28	27	8	10	24–30	30–64	27	27	2.0	2.0
LTS11	108	130	7	10	100–114	92–105	109	121	4.0	9.6
LTAS11	26	N/A	9	N/A	25–30	49	27	N/A	1.7	N/A

Numbers, where inserted, indicates segment number

lat Lateral accessory tubule, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lvs* lateroventral spine, *md* middorsal spine, *msw* maximum sternal width, *n* number of measured specimens, *s1–s11* segment lengths of trunk segments 1–11, *sd* standard derivation, *sw* standard width, *tl* trunk length, *vlt* ventrolateral tubule

Table 6 Summary of nature and location of sensory spots, glandular cell outlets, spines and tubules arranged by series in *Echinoderes riceae* n. sp

Segments	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	gco1		ss	ss					gco1	
2	gco1			ss					tu	ss, gco1
3	gco1		ss							gco1
4	ac	gco1								gco1
5	gco1		ss		ss			tu		ss, gco1
6	ac	gco1, ss			ss					ss, gco1
7	gco1		ss		ss			ac		gco1
8	ac	gco1, ss					tu	ac		gco1
9		gco1, ss	ss	ss		sp		ac	ss	gco1
10	gco1		ss	tu					ss	gco1
11		ss					ltas	lts		

ac Acicular spine, *gco1* glandular cell outlet type 1, *la* lateral accessory, *ld* laterodorsal, *ltas* lateral terminal accessory spine, *lts* lateral terminal spine, *lv* lateroventral, *md* middorsal, *ml* midlateral, *pd* paradorsal, *sd* subdorsal, *sl* sublateral, *sp* sieve plate, *ss* sensory spot, *tu* tubule, *vl* ventrolateral, *vm* ventromedial

the posterior side of the sensory spot, with usually one very long seta or spine pointing backwards and reaching far beyond the sensory spot limit (Fig. 10f). Cuticular hairs on this and the following segments quite long, emerging from bracteated perforation sites. Cuticular hair pattern extending from the midventral area toward the tergal–sternal junctions; a small area of cuticular hairs without perforation sites is present paraventrally (Fig. 10d). Tergal plates with a median belt of perforation sites (Fig. 7b). Pectinate fringe slightly longer than on previous segment.

Segment 3 consists of one tergal and two sternal plates as do the remaining segments to the 11th. (Figs. 7a, 9b). Spines or tubules are not present. A pair of sensory spots is present in a subdorsal position. Cuticular hair pattern and pectinate fringe as on previous segment.

Segment 4 with a middorsal spine (Figs. 7b, 9a, 10c), no sensory spots present, cuticular hair pattern similar to that of previous segments. Pectinate fringe well developed.

Segment 5 with one pair of lateroventral tubules (Figs. 7a, 9b, c, 10e). Paired sensory spots in ventromedial, subdorsal and midlateral positions. Cuticular hair patterns and pectinate fringe are similar to the preceding segments.

Segment 6 with a middorsal spine (Figs. 7b, 9a, 10c). No lateroventral spines or tubules present (Figs. 7a, 9c, 10e). Paired ventromedial, paradorsal and midlateral sensory spots. Cuticular hair pattern and pectinate fringe as in segment 5.

Segment 7 with one pair of lateroventral spines (Figs. 7a, 9c, 10e). Paired subdorsal and midlateral sensory spots. Cuticular hair patterns and pectinate fringe as in preceding segments.

Segment 8 with a middorsal spine (Figs. 7b, 9a), one pair of lateroventral spines, and lateral accessory tubules slightly separated from the lateroventral spines (Figs. 7a, 9d, 10e). A pair of paradorsal sensory spots present. Cuticular hair pattern and pectinate fringe similar to that of previous segments.

Segment 9 with one pair of lateroventral spines (Figs. 7a, 9b, d). Paired sensory spots present in ventrolateral, laterodorsal, subdorsal and paradorsal positions. Paired protonephridial openings present in sublateral positions; those openings are not sieve-plate-like, but are formed by a small opening surrounded by few pores and several papillae (Figs. 9d, 10f). Cuticular hair pattern and pectinate fringe similar to that of previous segments.

Segment 10 without spines. Males and females with a pair of laterodorsal tubules (Figs. 7b, d, 9a). Paired ventrolateral and subdorsal sensory spots present, the latter with a long conspicuous hair emerging (Fig. 10h). The posterior margin of the segment has a free flap with a midventral extension covering partially segment 11. The primary pectinate fringe is very short ventrolaterally, but increases in length toward the midventral position. Cuticular hair pattern more widely separated dorsally, and restricted to slightly smaller area ventrally.

Segment 11 with one pair of long and flexible lateral terminal spines (Figs 7a, b, 10g). Paradorsal sensory spots are present adjacent to the middorsal fringed area of the tergal plates. No cuticular hairs with perforation sites noted; short pectinate fringe present. Tergal extensions are pointed and with a small notch on the medial side (Figs. 7a, b, 9f, 10g, h). Their margins have cuticular hair-like extensions and fringes that are very long midlaterally and anterior to the insertion of the lateral terminal spines (Fig. 10g, h). Sternal plates short and triangular with fringed margins. Females with paired lateral terminal accessory spines (Figs. 7a, b, 9f, 10g); males with three pairs of

penile spines (P1–P3) instead. P1 and P3 longer than P2 which is shorter and many times thicker (Figs. 7c, d, 9d, g, 10h, i).

Remarks

Echinoderes riceae n. sp. is one of 12 species in the genus having middorsal spines on segments 4, 6 and 8. From these, 6 species (*Echinoderes abbreviatus* Higgins 1983; *Echinoderes higginsi* Huys and Coomans 1989; *Echinoderes hispanicus* Pardos et al. 1998; *Echinoderes kristenseni* Higgins 1985; *Echinoderes riedli* Higgins 1978; and *Echinoderes wallaceae* Higgins 1983) share the presence of lateroventral tubules on segment 2 and lateroventral spines on segments 6–9 with a lateral accessory tubule on segment 8 (see Higgins 1978, 1983, 1985; Huys and Coomans 1989; Pardos et al. 1998). However, *E. riceae* n. sp. shows a unique spine formula lacking the lateroventral spine on segment 6; thus, it is easily discriminated from all other similar species. Another relevant feature is the nearly complete absence of cuticular hairs on segment 1. Except for the cuticular hairs associated with the dorsal sensory spots, the surface of this segment is ventrally smooth giving it a special appearance only shared by few echinoderid species such as *Echinoderes coulli* Higgins 1977. This species show great differences in spine/tubule formula with *E. riceae* lacking middorsal or lateroventral spines and only showing lateroventral tubules on segments 5 and 8 (see Higgins 1977); therefore, there is no way to confuse them. However, the presence of cuticular hairs is a feature that has to be used with caution because not all species described (mostly old descriptions) contain this information in detail.

Three other kinorhynchs of this genus are known from the area, *E. adrianovi* n. sp., *E. horni* and *E. spinifurca*. As noted earlier, *E. adrianovi* n. sp. have middorsal spines on segments 4–8, lateroventral spines on segments 6–9, subdorsal and ventrolateral tubules on segment 2 and lateral accessory tubules on segment 8. *E. horni* has no middorsal spines, and *E. spinifurca*, although sharing its spine formula with 11 other species, has remarkably long terminal tergal extensions.

Introvert

Echinoderes is the most diverse genus within the phylum Kinorhyncha containing to date 77 species (Neuhaus 2012); unfortunately, only 6 of them have a detailed description of the introvert: *Echinoderes applicitus* Ostmann et al. 2012; *Echinoderes capitatus* Zelinka 1928; *Echinoderes cernunnos* Sørensen et al. 2012; *Echinoderes microaperturus* Sørensen et al. 2012, *E. spinifurca* and *E. tchefouensis* Lou 1934 (Nebelsick 1993; Ostmann et al. 2012; Sørensen and Pardos 2008; Sørensen et al. 2012b). Regarding the introvert structure, the echinoderid species with the greatest resemblance with *E. riceae* n. sp is *E. applicitus* showing identical uneven sectors and even sectors 4 and 8. The only differences are the number and distribution of leaf-like scalids in sectors 2 and 10 and the lack of a spinoscalid in ring 06 of sector 6. Despite the similarities showed by the introvert characters in *E. riceae* n. sp and *E. applicitus*, trunk features are completely different. Another species, but from a different genus, showing a similar scalid arrangement to *E. riceae* n. sp is *Meristoderes macracanthus* Herranz et al. 2012 (see Herranz et al. 2012); differences occur with respect to the number of scalids in the posteriormost rings and in *M. macracanthus*, its lack of leaf-like lateral scalids on sectors where trichoscalids are present (2, 4, 5, 7, 10). This feature makes the arrangement of scalids within the sector asymmetrical; a trait which is also shared by *E. riceae* n. sp, *E. applicitus, E. microaperturus*, *E. cernunnos*, *E. tchefouensis* in sectors 5 and 7 as well as *Meristoderes herranzae* Sørensen et al. 2012 (see Sørensen et al. 2012a) in sectors 2, 4, 8, 10, although the last four species have less spinoscalids in even sectors.

The scalid pattern of *E. riceae* n. sp. is unique. However, the arrangement of scalids by sectors with "double diamonds" in uneven sectors and "quincunxes" in the even ones (Fig. 8) seems to be rather common in the echinoderid genera *Echinoderes, Cephalorhyncha* and *Meristoderes*, and also in other genera such as *Antygomonas*, *Dracoderes*, *Centroderes* or *Pycnophyes*. The number of scalids of *E. riceae* n. sp (115) seems to be the highest ever reported, showing more scalids in the uneven numbered sectors. The presence of more scalids in the uneven sectors was previously reported by Sørensen et al. (2012b) as a common feature in the majority of species among Kinorhyncha. Nevertheless, the taxonomic and phylogenetic significance of the introvert characters, mainly type, number and arrangement of scalids, need to be tested and evaluated thoroughly, and thus, the resemblances reported should be used with caution.

Pycnophyes norenburgi n. sp. (Figs. 11, 12, 13; Tables 7, 8)

Order Homalorhagida (Zelinka 1896) Higgins 1964
Family Pycnophyidae Zelinka 1896
Genus *Pycnophyes* Zelinka 1907

Diagnosis

Pycnophyes with middorsal elevations on segments 1–9 flanked by paradorsal sensory spots. These sensory spots are associated with butterfly-like intracuticular atria on

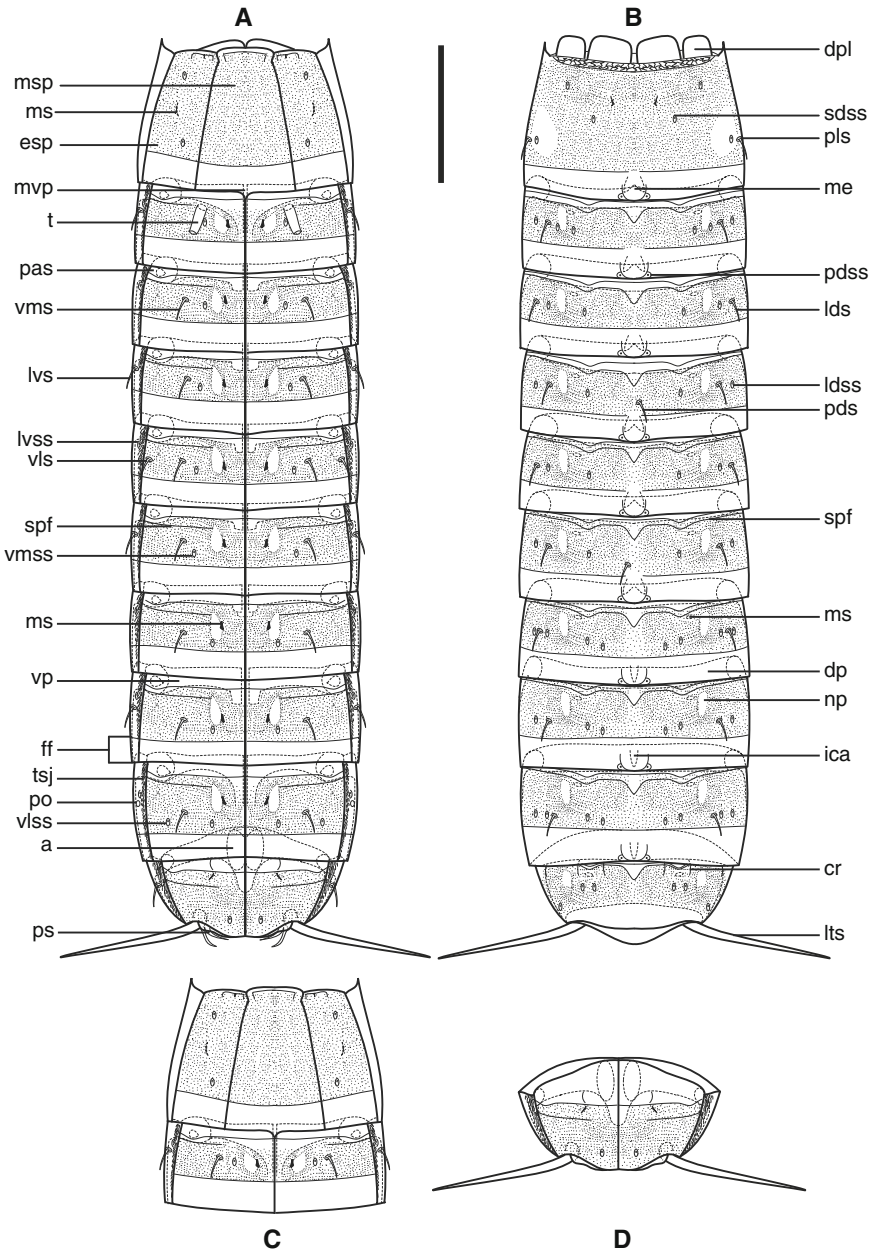

Fig. 11 Line art illustrations of *Pycnophyes norenburgi* n. sp. **a** Male, ventral view. **b** Male, dorsal view. **c** Female, segments 1–2, ventral view. **d** Female, segments 10–11, ventral view. *a* apodeme, anteromesial thickenings of ventral pachycyclus, *cr* cuticular ridge, *dp* dorsal pachycycli, *dpl* dorsal placid, *esp* episternal plate, *ff* free flap, *ica* intracuticular atria of sensory spot, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lts* lateroterminal spine, *lvs* lateroventral seta, *lvss* lateroventral sensory spot, *me* middorsal elevation, *ms* muscular scar, *msp* midsternal plate, *mvp* midventral process, *np* naked patch, *pas* peg and socket joint, *pds* paradorsal seta, *pdss* paradorsal sensory spot, *pls* paralateral seta, *po* protonephridial opening, *ps* penile spine, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *t* tube, *tsj* tergosternal junction, *vms* ventromedial seta, *vmss* ventromedial sensory spot, *vls* ventrolateral seta, *vlss* ventrolateral sensory spot, *vp* ventral pachycyclus. *Scale bar* 100 μm

segments 1–6 and with middorsal intracuticular atria on segments 7–9. Middorsal elevations less conspicuous on segments 7–9. Unpaired paradorsal seta on segments 4 and 6. Laterodorsal setae on segments 2–9, paralateral ones on segment 1, lateroventral ones on segments 2, 4, 6, 8 and 10 (the latter only in males), ventrolateral ones on segment 5 and ventromedial ones on segments 2–9 (absent on segment 2 in males). Conspicuous elongated paraventral muscular scars on segments 2–10, only visible with LM. Type 1 sensory spots in paradorsal positions on segments 1–9; any other sensory spot belongs to type 2. Trunk surface appears finely perforated in LM.

Etymology

This species is named in honor of Dr. Jon Norenburg, head of Zoology Department at the National Museum of Natural History, Smithsonian Institution, Washington DC, for his continuous contribution and support of meiofauna research worldwide.

Type material

Thirteen specimens (4 males and 9 females) mounted in Fluoromount G® were studied with LM, and 4 specimens

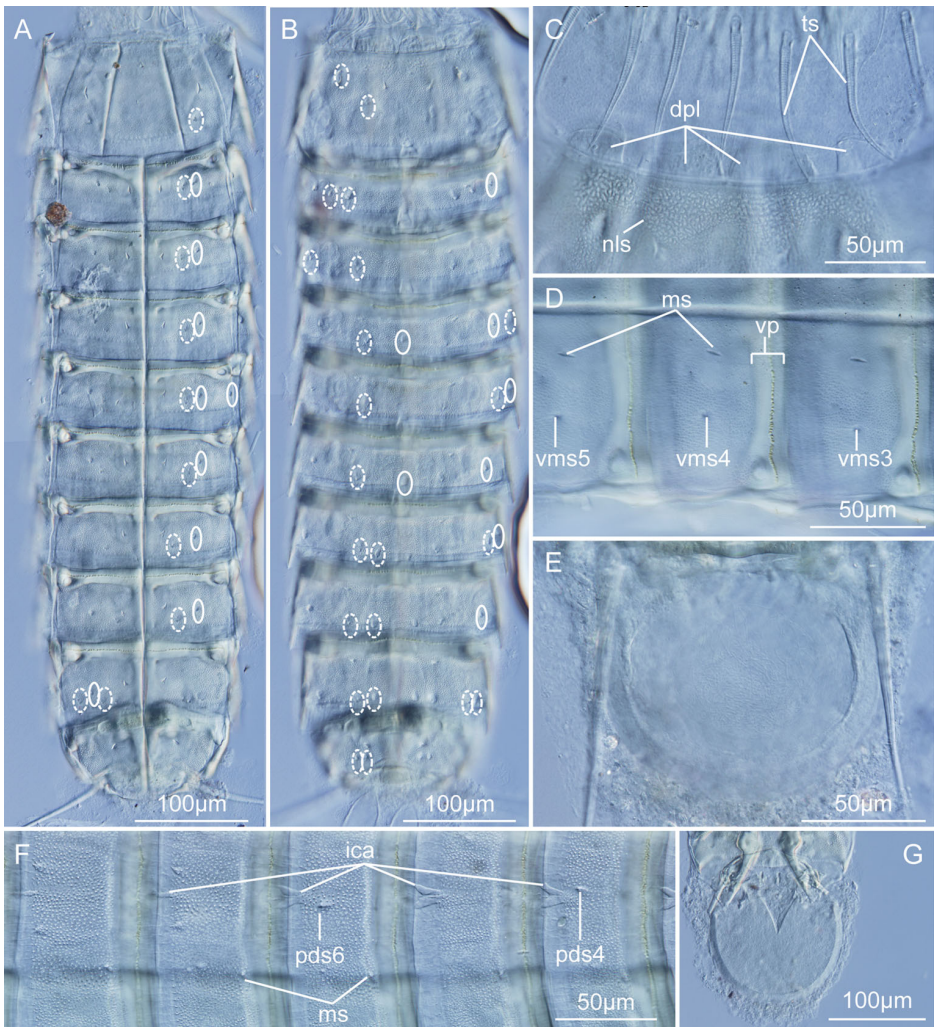

Fig. 12 Light micrographs showing details in neck and trunk morphology of *Pycnophyes norenburgi* n. sp. **a** Allotypic female, USNM 1207884, ventral view. **b** Allotypic female, USNM 1207884, dorsal view. **c** Male, dorsal placids. **d** Male, sternal plates of segments 3–5. **e** Female, spermatophore with a tangled ball of sperm inside. **f** Holotypic male, USNM X, dorsal view of segments 4–8. **g** Female, spermatophore overview attached to the last segment of the trunk between the two laterotermiinal spines. Sensory spots are marked with dotted circlets. Setae are marked with *circlets*. *dpl* dorsal placids, *ica* intracuticular atria of sensory spot, *ms* muscular scar, *nls* net-like structure, *pds* paradorsal seta, *ts* trichoscalid, *vms* ventromedial seta, *vp* ventral pachycyclus. Digits after abbreviations refer to segment numbers

(3 males and 1 female) were studied with SEM. Holotype: adult male collected on August 2011 at the 20 miles station, Fort Pierce, off the Floridian West coast (Fig. 1) 27°30,84′N 79°54,86′ W; 152 m depth from fine mud, deposited at the National Museum of Natural History (Smithsonian Institution) under accession number USNM 1207950. Allotype: adult female, same collecting data as holotype, deposited at the National Museum of Natural History under accession number USNM 1207884. Paratypes: 1 male and 4 females, same collecting data as holotype, deposited at the National Museum of Natural History under accession numbers USNM 1207885–1207889. The remaining material is deposited in the Meiofauna Laboratory collection at the Facultad de Biología, Universidad Complutense de Madrid, accession numbers K15/37-43.

Additional material

Because of the close resemblance of the new species with *Pycnophyes frequens*, topotype specimens of this latter species were loaned from the Smithsonian Institution and checked with LM. This material includes two males and two females collected by R. P. Higgins on June 13, 1962, in Salisbury Cove, Mount Desert Island, Maine, position 44°26′N, 68°17′W; 15 m depth, gray mud, deposited in the

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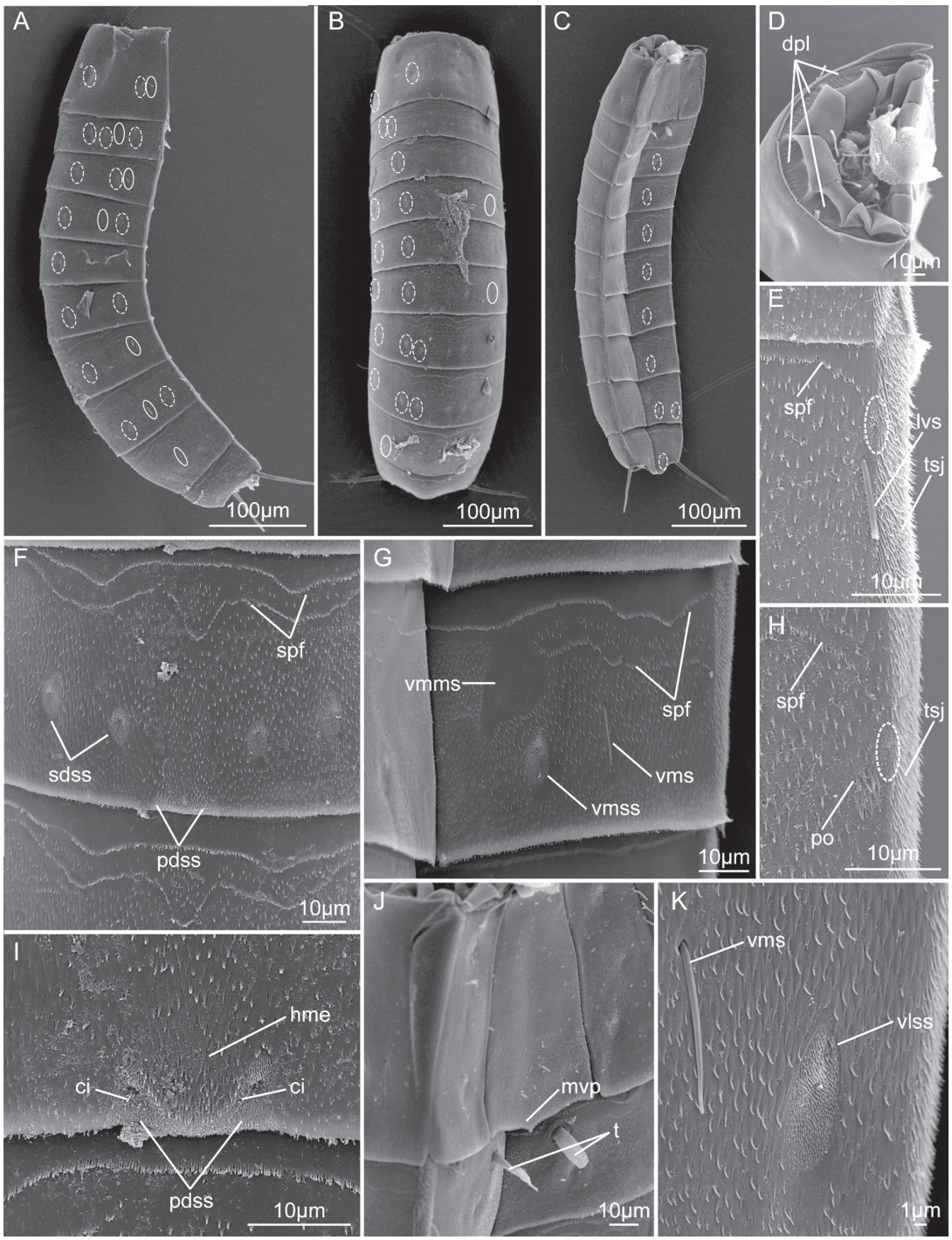

Fig. 13 Scanning electron micrographs showing trunk morphology and cuticular details in *Pycnophyes norenburgi* n. sp. **a** Male, lateral view. **b** Male, dorsal view. **c** Male, lateroventral view. **d** Male, dorsal placids. **e** Male, lateral view of segment 4. **f** Male, dorsal view of segment 7. **g** Male, ventral view of segment 7. **h** Male, lateral view of segment 9, protonephridial opening. **i** Male, dorsal view of segment 6. **j** Male, lateroventral view of segments 1–2. **k** Male, ventral view of segment 9. *ci* cilium, *dpl* dorsal placid, *hme* hairy middorsal elevation, *lvs* lateroventral seta, *mvp* midventral process, *pdss* paradorsal sensory spot, *po* protonephridial opening, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *t* tube, *tsj* tergosternal junction, *vmms* ventromedial muscular scar, *vms* ventromedial seta, *vmss* ventromedial sensory spot, *vlss* ventrolateral sensory spot. Sensory spots are marked with *dotted circlets*. Setae are marked with *circles*

Table 7 Measurements (in µm) for adults of *Pycnophyes norenburgi* n. sp

Character	*n*		Range		Average		SD	
	♀	♂	♀	♂	♀	♂	♀	♂
TL	5	4	601–669	646–738	635	710	28.1	42.9
SW10	3	1	129–141	129	135	–	8.9	–
SW/TL (%)	3	1	19–23	0.18	0.21	–	0.03	–
MSW-8	3	1	151–152	155	152	–	0.03	–
MSW/TL (%)	3	1	0.23–0.25	0.21	0.24	–	0.02	–
LTS/TL	3	2	0.16–0.19	0.17	0.17	0.17	0.02	0.01
S1	3	1	103–109	104	107	–	4.1	–
S2	3	1	62–67	68	64	–	3.1	–
S3	3	1	64—69	63	66	–	3.8	–
S4	3	1	58–70	70	64	–	8.7	–
S5	3	1	59–68	69	63	–	5.9	–
S6	3	1	63–71	74	67	–	5.9	–
S7	3	1	69–70	78	69	–	1.3	–
S8	3	1	74–77	81	76	–	2.5	–
S9	3	1	67–73	80	70	–	4.1	–
S10	3	1	70–78	86	74	–	5.8	–
S11	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LTS11	4	2	108–122	120–133	115	125	7.2	0.04
SPL	5	0	97–125	–	108	–	13.3	–
SPW	5	0	103–136	–	121	–	13.3	–
SWT	5	0	8–13	–	11	–	1.7	–

lts Lateral terminal spine, *msw* maximum sternal width, *n* number of measured specimens, *sd* standard deviation, *spl* spermatophore length, *spw* spermatophore width, *sw* standard width, *s1–s11* segment lengths of trunk segments 1–11, *swt* spermatophore wall thickness, *tl* trunk length

marine invertebrate collection of US National Museum, under accession numbers W30973–W30974 and 1185888–1185889. Loaned specimens were in poor condition and were subsequently remounted in glycerin–paraffin (two specimens) and Fluoromount G® (two specimens). The specimen under accession number 1185888, male, was assigned as neotype. Moreover, seven specimens of *P. frequens* collected from Woods Hole (Massachusetts) loaned for examination from the Natural History Museum of Denmark (accession numbers ZMUC KIN-00667 to KIN 00673).

Description

Measurements and dimensions of the examined specimens are summarized in Table 7. The distribution of cuticular setae and sensory spots is summarized in Table 8.

Mouth cone and introvert armature could not be examined in detail in any of the prepared specimens.

Neck with 4 dorsal and 2 ventral placids. All placids seem robust with a concave surface. Medial two dorsal placids wide and rectangular, whereas the lateral two dorsal placids are narrower, with a square profile (Figs. 11b, 12c, 13d). Ventral placids smaller than dorsal ones. Ventral placids widely rectangular, extending from the midventral line to the middle region of the episternal plates. All placids without trichoscalid plates, joined and articulated with the anterior edge of the first trunk segment. Placids continue anteriorly and merge with the thin and flexible cuticle of the introvert.

Trunk with 11 segments (Figs. 11a, b, 12a, b, 13a, c). First segment with one tergal and three ventral plates. Remaining segments with one tergal and two sternal plates. The segment width is fairly constant, with the maximum width at segment 8, turning narrower posteriorly. Cuticle appears thick, pachycycli and peg and socket joints appear well developed on all segments, the latter less conspicuous on segment 10. Most trunk surface, except pairs of naked oval patches located laterodorsally and ventromedially, covered by short, scale-like cuticular hairs without perforation sites (Figs. 11a, b). These cuticular hairs are longer on the middorsal elevation and on the first half of the segment than on the posterior one. In LM, the cuticle appears finely punctuated (Fig. 12f), probably perforated by numerous intracuticular pores. However, these do not reach the surface that appears smooth with SEM, except for the scale-like hairs referred to above (Figs. 13f, g). Tergal plates of segments 1–6 with paradorsal sensory spots

Table 8 Summary of nature and location of cuticular features arranged by series for adults of *Pycnophyes norenburgi* n. sp

f female condition of sexually dimorphic character, *ld* laterodorsal, *lts* laterotermial spine, *lv* lateroventral, *m* male condition of sexually dimorphic character, *p* penile spine, *pd* paradorsal, *pl* paralateral, *sd* subdorsal, *se* setae, *ss* sensory spot, *t* tube, *vl* ventrolateral, *vm* ventromedial, *1* unpaired cuticular structure

Segments	PD	SD	LD	PL	LV	VL	VM
1	ss	ss	ss, ss	se			ss, ss
2	ss	ss	ss, se, ss		se, ss		ss, se(f), t(m)
3	ss	ss	ss, se		ss		ss, se
4	se(1), ss	ss	se, ss		se, ss		ss, se
5	ss	ss	ss, se		ss	se	ss, se
6	se(1), ss	ss	se, ss		se, ss		ss, se
7	ss	ss, ss	ss, se, ss		ss		ss, se
8	ss	ss, ss	se, ss		se, ss		ss, se
9	ss	ss, ss	se(m), ss, ss		ss	ss	ss, se
10		ss, ss	ss		se(m)		ss
11					lts		

associated with conspicuous butterfly-like intracuticular atria; middorsal elevations never surpasses the posterior edge of the segment (Figs. 12f, 13f, i). Tergal plates of segments 7–9 with inconspicuous middorsal elevations and middorsal unpaired intracuticular atria, not butterfly-like (Figs. 11b, 12f, 13f). Two pairs of muscular scars, one conspicuous and elongated in paraventral position and one more rounded, oblique and less clear, in subdorsal position (Fig. 12d). Free flaps striated longitudinally, only visible with LM. Minute pectinate fringe, only visible with SEM. Secondary pectinate fringes located in the anteriormost region of the segments.

Segment 1 with lateral margins of tergal plate projecting anteriorly into horn-like extensions. Tergal anterior edge of segment smooth, not denticulated, followed by a net-like area, not always well developed (Fig. 12c). Tergal plate with middorsal elevation flanked by one pair of paradorsal sensory spots associated with intracuticular atria. One pair of paralateral setae and three additional pairs of sensory spots, one subdorsal and two laterodorsal. From these latter, one is located near the anterior margin of the segment, and the other is very close to the already mentioned paralateral setae. All sensory spots on this and the following segments (except the paradorsal ones), belong to type 2. They are oval and elongated, with two pores, median and anterior, surrounded by numerous cuticular papillae. A cilium emerges from the median pore, while the anterior pore opens on a short elevated tube. The paradorsal sensory spots share an elongated area of cuticular papillae. Their cuticular papillae are smaller, surrounding a single pore through which a cilium emerges (Fig. 13i). Ventral side with one trapezoidal midsternal plate and two episternal plates, all of them with an inconspicuous cuticular ornamentation in its anterior region, weakly concave. Posterior margin of midsternal plate with a pointed midventral process (Fig. 13j). Each episternal plate with a curved, crescentic muscular scar in the middle region of the plate and two ventromedial sensory spots, one located at the anterior third of the segment, and one at the posterior third. Pectinate fringe on the posterior edge of the segment reduced, only detectable with SEM.

Segment 2 showing a tergal plate with a middorsal elevation flanked by one pair of paradorsal sensory spots with conspicuous intracuticular atria. One pair of laterodorsal and lateroventral setae. Four additional pairs of sensory spots located in the middle region of the tergal plate: one subdorsal, two laterodorsal and one lateroventral. One of the laterodorsal sensory spots appears mesial to the laterodorsal seta and the other one laterally, close to the paralateral line (Fig. 11b). Lateroventral sensory spots are smaller and narrower than others and appear located adjacent to the tergosternal junctions (Fig. 13e, h). One pair of subdorsal oval muscular scars, inconspicuous and only visible with LM, located anterior to the subdorsal sensory spots, between this sensory spot and one laterodorsal-naked oval patch. Two secondary pectinate fringes run parallel and close to the anterior edge of the segment (Fig. 13f). The anteriormost one shows two subdorsal indentations pointing backwards, while the posterior one has three similar indentations, two subdorsal and one middorsal, this latter deeper and pointed (Figs. 11b, 13f). Sternal plates with a pair of ventromedial sensory spots and a pair of paraventral elongated muscular scars. A pair of ventromedial naked oval patches appears adjacent to the muscular scars. Females furthermore with a pair of ventromedial setae (Fig. 11c). Males with one pair of ventromedial tubes, big and short (Figs. 11a, 13j). Longitudinal band of densely packed cuticular hairs along the tergosternal junctions (Figs. 13e, h). Two secondary fringes near and parallel to the anterior margin of the segment. The anteriormost extends along the whole ventral margin of the segment and shows ventrolateral indentations. The posteriormost without indentations and running from the tergosternal junctions to the ventromedial naked patches. Pectinate fringe as on previous segment.

Segment 3 showing a tergal plate with middorsal elevation, one pair of paradorsal sensory spots associated with intracuticular atria similar to those on previous segments. One pair of laterodorsal setae located more laterally than the same on previous segment. Three additional pairs of sensory spots, one subdorsal, one laterodorsal and one lateroventral. The laterodorsal pair of sensory spots appears in the same position as the laterodorsal setae of segment 2 (Figs. 11b, 12b, 13a). Lateroventral sensory spots at the same position as those on the previous segment. One pair of subdorsal muscular scars located anterior to the subdorsal sensory spots and one pair of laterodorsal-naked oval patches. Sternal plates with one pair of ventromedial setae located slightly anterior to the sensory spots (Fig. 11a).

Segment 4 showing a tergal plate with a middorsal elevation, one pair of paradorsal sensory spots associated with intracuticular atria similar to those on previous segments. A single, unpaired paradorsal seta on the right side of the middorsal elevation (Figs. 11b, 12f). One pair of laterodorsal and lateroventral setae, the former appears in the same position as the laterodorsal sensory spots of segment 3 (Figs. 11b, 12b, 13a). Three pairs of sensory spots, one subdorsal, one laterodorsal and one lateroventral, the latter same as on previous segments (Fig. 13e). Laterodorsal sensory spots appear in the same position as the laterodorsal setae of segment 3 (Fig. 13a). One pair of subdorsal muscular scars and one pair of laterodorsal-naked oval patches as those on segment 3. Sternal plates as on segment 3 (Figs. 11a, 12d).

Segment 5 with tergal and sternal plates as those on segment 3 but with a pair of ventrolateral setae.

Segment 6 with a tergal plate similar to that on segment 4 but with the single, unpaired paradorsal seta on the left side of the middorsal elevation (Figs. 11b, 12f, 13i). Sternal plates as those on segment 4.

Segment 7 showing a tergal plate with middorsal elevation less conspicuous than those on previous segments. Middorsal intracuticular atria pointed, not butterfly-like (Fig. 12f). One pair of laterodorsal setae. Six pairs of tergal sensory spots, one paradorsal, two subdorsal (Fig. 11b, 13f), two laterodorsal, on both sides of the laterodorsal seta; and one lateroventral same as on previous segments. Sternal plates similar to those on segment 3, with the ventromedial sensory spots mesially displaced (Figs. 11a, 12a, 13b, g).

Segment 8 showing a tergal plate with middorsal elevation, intracuticular atria and paradorsal sensory spots similar to those on segment 7. Without paradorsal setae (Fig. 12f). Pairs of laterodorsal and lateroventral setae, the former located in the same position as the mesial laterodorsal sensory spots of segment 7 (Figs. 11b, 12b, 13a). Four additional pairs of sensory spots: two subdorsal, one laterodorsal in the same position as the laterodorsal setae of segment 7, and one lateroventral same as on previous segments (Figs. 12b, 13a). Sternal plates as on segment 7.

Segment 9 with tergal plate same as on segment 8 but with an additional pair of laterodorsal sensory spots. Laterodorsal setae present in males only. Protonephridial opening located paralaterally, not sieve-like but formed by several minute tubes with bevelled endings and surrounded by few short cuticular hairs (Fig. 13h). Sternal plates similar to those on segment 7 but with one pair of ventrolateral sensory spots (Figs. 11a, 13c, k).

Segment 10 showing a tergal plate without middorsal elevation or intracuticular atria. One pair of lateroventral setae present in males only (Figs 11a, d). With one pair of subdorsal muscular scars and three pairs of sensory spots: two subdorsal and one laterodorsal, the latter near the posterior margin of the segment (Fig. 12b). Three pairs of longitudinal cuticular ridges near anterior margin of the segment, two subdorsal and one laterodorsal. Sternal plates with one pair of ventromedial sensory spot near the posterior margin of the segment (Fig. 13c) and one pair of elongated ventromedial muscular scars similar to those on previous segments but reversed and more oblique. One pair of anteromedial apodemes near anterior margin. Females with the apodemes displaced. One pair of ventromedial cuticular ridges. Posterior margin of the segment rounded and moderately projecting ventromedially (Fig. 13c).

Segment 11 showing tergal and sternal plates without any remarkable cuticular features, only a pair of laterotterminal cuticular processes on the tergal plate and one pair of robust laterotterminal spines. Males with two pairs of flexible penile spines of equal size. Some of the studied female specimens bear spermatophores, they are spherical capsules covered by debris, tightly held at the posterior end of the trunk, between the two laterotterminal spines that appear to embrace it (Figs. 12e, g). These spermatophores show a thick wall slightly wider in the middle region than in the lateral areas (Fig. 12e). Measurement data on these spermatophores are included in Table 7. In all cases, the capsules contained a tangled ball of sperm inside without any recognizable arrangement.

Remarks

The new species shares the presence of paradorsal setae exclusively on segments 4 and 6 with only two species: *Pycnophyes cryopygus* Higgins and Kristensen 1988 and *Pycnophyes emarginatus* Higgins 1983. However, *P. cryopygus* presents laterodorsal setae on segments 2, 4, 6–9, whereas the new species has this kind of setae on segments 2–9. Moreover, *P. cryopygus* differs from the new species by the presence of middorsal processes and lateroventral setae on segments 2–4, 6, 8 and 10. Although *P. cryopygus* and the new species have

ventromedial setae on segments 3–9, *P. cryopygus* has these setae on segment 3–6 lined-up and the setae on segment 7–9 are mesially displaced, whereas the new species shows always all of them lined-up, exactly in the same point on each segment. Finally, the distribution of sensory spots and muscular scars in both dorsal and ventral sides is different in both species (Higgins and Kristensen 1988).

Pycnophyes emarginatus and *Pycnophyes norenburgi* n. sp. share the presence of middorsal setae on segments 4 and 6, laterodorsally on segments 2–8 and ventromedially on segments 3–9 (Higgins 1983). However, *P. emarginatus* lacks laterodorsal setae on segment 9 and ventrolateral ones on segment 5, and it also differs from the new species regarding the position of the ventromedial setae with relative to the sensory spots (these setae appear mesial to sensory spots) and the distribution of lateroventral setae and sensory spots (Higgins 1983).

Nevertheless, *P. norenburgi* n. sp. shows most resemblance with *P. frequens*, described from the East coast of USA and redescribed by Higgins (1965). From the information provided in the original description (Blake 1930) and the redescription (Higgins 1965), the distribution pattern of sensory spots and setae cannot be stated reliably. Consequently, the authors requested both topotype material and recently collected specimens from Woods Hole (Massachusetts). Examination of this material allowed a proper identification of the relevant cuticular structures and their position. The protuberances identified by Higgins (1965) as "sensory hair" on segment 5 in ventrolateral position as well as the middorsal ones on segments 4, 6 and 8 correspond to setae (the latter are actually located in paradorsal position, not middorsally). Contrarily, the protuberances observed ventrolaterally on the episternal plates and along the tergal plate of segment 10 are actually sensory spots and not setae. *P. frequens* and the new species described herein share many similarities: the finely perforated trunk surface; the ventral cuticular ornamentation on segment 1; shape and position of paraventral muscular scars (segments 2–10); the naked oval patches next to these paraventral muscular scars, the appearance of pachycycli, peg and socket joints and anteromesial apodemes; the middorsal elevations on segments 1–9 associated with intracuticular atria, the unpaired paradorsal setae, laterodorsal setae on segments 2–9, lateroventral ones on segments 2, 4, 6, 8 and 10 (the latter only in males), ventrolateral ones on segment 5 and ventromedial ones on segments 3–9 (males also with a pair of setae on segment 2). Nevertheless, *P. frequens* bears paradorsal setae on segments 4, 6 and 8, lateroventral ones on segment 9 and laterodorsal ones on segment 9 in both sexes, whereas *P. norenburgi* n. sp. lacks paradorsal setae on segment 8 and lateroventral ones on segment 9, as well as laterodorsal ones on segment 9 in females. Moreover, despite both species show laterodorsal sensory spots not aligned (sensory spots of segments 3, 5 and 7 appear slightly mesially displaced), all the specimens of *P. norenburgi* n. sp. have alternation as to the position between laterodorsal setae and sensory spots of these three segments, so neither laterodorsal setae nor sensory spots appear aligned, while *P. frequens* has the laterodorsal setae lined-up. Furthermore, the tergal anterior ornamentation of the first segment with a net-like pattern in *P. norenburgi* n. sp. lacking it in *P. frequens*, the number and shape of secondary pectinate fringes, and the distribution of longitudinal cuticular ridges are different in both species. All these characters were consistently found in all the specimens studied from Florida and support the erection of the new species *P. norenburgi* n. sp.

The presence of specimens bearing a spherical capsule with sperm inside attached to the segment 11 is not new; such structure, identified as spermatophore, was observed by several authors in different kinorhynch species, most of them homalorhagids: *Centroderes spinosus*, *Kinorhynchus ilyocryptus* (Higgins 1961), *K. langi*, *Kinorhynchus phyllotropis* Brown and Higgins 1983, *Pycnophyes dentatus* (Reinhard 1881), *Pycnophyes flaveolatus* Zelinka 1928, *P. frequens*, *Pycnophyes greenlandicus* Higgins and Kristensen 1988 and *Pycnophyes kielensis* Zelinka 1928 (see Nyholm 1947; Higgins 1965, 1974; Brown 1983; Kristensen and Higgins 1991; Adrianov and Malakhov 1999; Neuhaus 2012). Data on number of individuals bearing such capsules is available only for *K. phyllotropis* (see Brown 1983), *P. dentatus* and *P. kielensis* (see Neuhaus 2012). The incidence detected was similar for these three species, 1 of 88 females, while in the present work, about 50 out of 100 collected specimens had this structure. However, only few specimens kept it attached after the fixing process, perhaps explaining the low incidence reported in the literature. In all cases, the spermatophores were observed attached to females, with sperm inside as a tangled ball suggesting that the sperm could have started the migration into the female body through the gonopores (Brown 1983).

However, the knowledge about these spermatophores and their function in reproduction is still limited, as well as how males produce the spermatophores, how these are transferred from males to females and how and where the fertilization occurs (Neuhaus 1999). Also, the role of the so-called penile spines of males and their special tubes of segment 2 is to be determined, as well as the female oviposition (Nyholm 1947; Lang 1963; Brown 1983; Kristensen and Higgins 1991; Neuhaus and Higgins 2002). Future studies, probably through culturing techniques, would shed light into the reproductive processes of kinorhynchs.

Conclusion

To date, studies in the Atlantic Floridian waters have reported a total of 7 species of kinorhynchs belonging to 4 different genera: *A. paulae*, *E. bookhouti*, *E. horni*, *E. spinifurca*, *T. seminoli*, *Z. brightae* and *Z. floridensis*. From these only *E. bookhouti* was not found in Fort Pierce. The present contribution adds four new species to the area: *A. gwenae* n. sp, *E. adrianovi* n. sp., *E. riceae* n. sp. and *P. norenburgi* n. sp. Very recent samplings in the region have revealed additional, yet undescribed kinorhynch species (Herranz pers. obs.), showing that the area still holds a high diversity of Kinorhyncha to unveil.

The 20 miles station seems to be a special locality; its particular type of sediment called "green/gray mud" shows a highly diverse kinorhynch fauna with 5 different genera and 6 different species: *Z. floridensis*, *Centroderes* sp. 1, *E. adrianovi* n. sp. *E. riceae* n. sp. *A. gwenae* n. sp. and *P. norenburgi* n. sp. All these species were found on this locality exclusively and are not present in nearby localities with different sediments. It should be noted that *Z. floridensis*, *E. riceae* and *P. norenburgi* n. sp. were also very abundant.

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Chapter V

Fissuroderes sorenseni sp. nov. and *Meristoderes boylei* sp. nov.: First Atlantic recording of two rare kinorhynch genera, with new identification keys

Fissuroderes sorenseni sp. nov. y *Meristoderes boylei* sp. nov.:
Primeras citas atlánticas de dos géneros de kinorrincos poco comunes,
aportando nuevas claves de identificación

MARÍA HERRANZ, Fernando Pardos
Zoologischer Anzeiger 253: 93– 111 (2013)

Este trabajo incluye la descripción de dos nuevas especies del filo Kinorrincos, recogidas en la costa oeste de Florida, USA en el Atlántico Occidental y asignadas a dos géneros recientemente descritos y aún poco conocidos: *Fissuroderes* Neuhaus and Blasche, 2006 y *Meristoderes* Herranz et al., 2012. Ambos géneros pertenecen a la familia Echinoderidae Bütschli, 1876. Ninguno de los dos géneros se había registrado previamente en aguas atlánticas. Esta publicación también proporciona las primeras imágenes de microscopía electrónica de barrido y una descripción detallada del introverto de una especie de *Fissuroderes*, que ha revelado nuevos caracteres de posible importancia filogenética. Además, se proporciona una clave dicotómica para todas las especies conocidas de ambos géneros discutiendo su distribución biogeográfica y su posición taxonómica.

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Fissuroderes sorenseni sp. nov. and *Meristoderes boylei* sp. nov.: First Atlantic recording of two rare kinorhynch genera, with new identification keys

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ABSTRACT

The present contribution includes the description of two new species of the phylum Kinorhyncha collected off the East coast of Florida, USA and assigned to two recently and still poorly known genera: *Fissuroderes* Neuhaus and Blasche, 2006 and *Meristoderes* Herranz et al., 2012. They both belong to the family Echinoderidae Bütschli, 1876. Species of *Fissuroderes* and *Meristoderes* have not previously been recorded from the Atlantic Ocean. This publication also provides the first SEM images and a detailed description of the introvert of a *Fissuroderes* species, revealing new characters of phylogenetic significance. A dichotomous key for all the known species of both genera is provided, together with discussions of their biogeographical distribution and taxonomical status.

1. Introduction

Kinorhynchs are marine meiobenthic ecdysozoans ranging from 0.1 to 1 mm body length that occupies different habitats, mostly muddy or sandy sediments, from the intertidal to the deep sea (Zelinka, 1928; Danovaro et al., 2002; Neuhaus and Higgins, 2002; Sørensen and Pardos, 2008; Neuhaus, 2012). Lately, the increase of extensive samplings vs. punctual random collections has improved the knowledge of kinorhynch diversity and distribution (Sánchez et al., 2012; Sørensen et al., 2012a, 2013). Currently the phylum comprises around 200 species distributed into 22 genera and 8 families. Within the last 10 years 6 new genera have been discovered: *Fissuroderes* Neuhaus and Blasche, 2006; *Meristoderes* Herranz et al., 2012; *Tubulideres* Sørensen et al., 2007; *Wollunquaderes* Sørensen and Thormar, 2010; *Triodontoderes* Sørensen and Rho, 2009 and *Franciscideres* Dal Zotto et al., 2013 suggesting that our knowledge of the diversity of Kinorhyncha is still far to be complete (see Neuhaus and Blasche, 2006; Sørensen et al., 2007; Sørensen and Rho, 2009; Sørensen and Thormar, 2010; Herranz et al., 2012; Dal Zotto et al., 2013).

Fissuroderes and *Meristoderes* are both recently described genera. They belong to the family Echinoderidae Bütschli, 1876, which comprises species with the cuticle of the first trunk segment forming a closed ring, the cuticle of second trunk segment with complete, incomplete or lacking midventral and lateral divisions while the cuticle of trunk segments 3–10 have midventral and lateral articulations resulting in two sternal plates and one tergal plate; lack of midterminal spine, and dorsal spines, if present, middorsally aligned (Neuhaus and Blasche, 2006; Herranz et al., 2012). The genus *Fissuroderes* comprises 5 species, four of them (*Fissuroderes higginsi* Neuhaus, 2006; *Fissuroderes novazealandia* Neuhaus, 2006; *Fissuroderes papai* Neuhaus, 2006 and *Fissuroderes rangi* Neuhaus, 2006) found near New Zealand and one (*Fissuroderes thermoi* Neuhaus and Blasche, 2006) from Pacific Costa Rica. Unfortunately none of the descriptions contained SEM information. The genus *Meristoderes* has originally been described from very distant areas such as the Mediterranean Sea (*Meristoderes macracanthus* Herranz et al., 2012) and the Pacific Ocean (*Meristoderes galatheae* Herranz et al., 2012). Currently it comprises six described species, of which the remaining four were found very recently also from Pacific waters: *Meristoderes elleae* Sørensen et al., 2013; *Meristoderes glaber* Sørensen et al., 2013; *Meristoderes herranzae* Sørensen et al., 2013; *Meristoderes imugi* Sørensen et al., 2013 and a yet undescribed species, *Meristoderes* sp. (see Sørensen et al., 2013).

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The present contribution includes the description of two new species belonging to *Fissuroderes* and *Meristoderes*, respectively, which constitute the first recordings of the genera in Atlantic waters. This publication also provides the first SEM images and a detailed description of the introvert of a *Fissuroderes* species, revealing new, phylogenetically significant characters. The new data will be analyzed to clarify the biogeography and taxonomical status of these still poorly known genera. A dichotomous key for all the known species of both *Fissuroderes* and *Meristoderes* is provided as well.

2. Materials and methods

Specimens of *Fissuroderes sorenseni* sp. nov. and *Meristoderes boylei* sp. nov. were collected 3 miles off Fort Pierce (Florida) in August and September 2011 and 2012, respectively. Sediment samples were taken from the research vessel RV Sunburst, owned and operated by the Smithsonian Marine Station at Fort Pierce (SMSFP), using a meiobenthic anchor dredge that only collects the uppermost part of the sediment. Kinorhynchs were extracted from the sediment following the bubbling technique of Higgins (Higgins, 1988; Neuhaus, 2003; Sørensen and Pardos, 2008) and fixed in 4% paraformaldehyde.

Specimens prepared for light microscopy (LM) were dehydrated through a graded series of ethanol and transferred to glycerine prior to mounting in Fluoromount G®. The specimens were examined and photographed using an Olympus BX51 microscope with differential interference contrast optics equipped with an Olympus Colorview DP70 camera. Measurements were made using Olympus Cell^ A software (Olympus, Europe). Specimens for SEM were dehydrated through a graded series of ethanol and critical point dried. The dried specimens were mounted on aluminum stubs, sputter coated with gold-palladium and imaged with either a HITACHI S4800 or a JEOL JSM 6335 field emission scanning electron microscope. Coating and SEM imaging were performed at the United States Department of Agriculture (USDA), United States Horticultural Research Laboratory in Fort Pierce, FL. Additional SEM imaging was performed at the ICTS Centro Nacional de Microscopía Electrónica, Universidad Complutense de Madrid, Spain. All specimens were ultrasonically cleaned by exposing them to ultrasound intervals of 5–10 s before the dehydration procedure.

3. Results

Order Cyclorhagida (Zelinka, 1896) Higgins, 1964
Family Echinoderidae Bütschli, 1876
Genus *Fissuroderes* Neuhaus and Blasche, 2006

3.1. *F. sorenseni* sp. nov. (*Figs. 1–5 and Tables 1 and 2*)

3.1.1. Diagnosis

Fissuroderes with four long middorsal spines on segments 4, 5, 6 and 8 increasing progressively in length toward the posterior segments; broad ventrolateral tubules on segment 2; wide lateroventral tubules on segment 5; lateroventral long and flexible spines on segments 6–9; laterodorsal tubules on segment 10. Tergal extensions of segment 11 elongated with pointed tips. Males showing two pairs of crenulated penile spines, females with a pair of lateral terminal accessory spines instead. Glandular cell outlets type 2 present in subdorsal positions on segment 2 and laterodorsal positions on segments 8 and 9. Females with additional glandular cell outlets type 2 in ventrolateral and ventromedial positions on segments 7 and 8.

3.1.2. Type material

Holotype: adult male collected on September 20, 2012 3 miles off Fort Pierce, Florida, 27°29.73′ N, 80°13.83′ W, at 11 m depth from muddy sand, mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark under accession number ZMUC KIN-694. Allotype: adult female collected on August 3, 2011, 3 miles off Fort Pierce, Florida, 27°29.78′ N, 80°13.73′ W, at 10 m depth from muddy sand, mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark under accession number ZMUC KIN-695. Paratypes: two adult males collected on the same date and location of the allotype mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark under accession numbers ZMUC KIN-696 and KIN-697.

3.1.3. Additional material

One male collected from the same location and on same date as the allotype and paratype, mounted in Fluoromount G®. Two females and one male collected from the same locality as the paratype, and two more females collected from the same locality as the holotype, all of them mounted for SEM. All additional material is stored in the Meiofauna Laboratory collection at the Universidad Complutense de Madrid under accession numbers K15/28-30.

3.1.4. Etymology

The species is named after Dr. Martin V. Sørensen, Natural History Museum of Denmark, colleague, friend and one of the current researchers with a major contribution to the knowledge of the phylum Kinorhyncha.

3.1.5. Description

Adults with head, neck and eleven trunk segments (Figs. 1 and 5A). Measurements and dimensions are given in Table 1. A summary of sensory spots, spines, tubules and glandular cell outlet positions is provided in Table 2.

The head consists of a retractable mouth cone and an introvert with seven rings of scalids (Figs. 2 and 4A–E). Outer armature of the mouth cone formed by nine outer oral styles alternating in size between 5 large well-developed ones situated according to uneven sectors of the introvert, and 4 small ones situated according to even sectors (Fig. 4C); middorsal outer oral style is missing. All outer oral styles divided into two subunits: an elongated, rectangular and smooth proximal part and a distal long thin and curved part terminating into a sharp tip (Fig. 4C). The size differences are due to the sizes of the basal style units that alternate between longer and shorter ones. Inner armature of the mouth cone could not be examined in detail.

The introvert has seven rings of scalids and one additional ring of trichoscalids that are associated with the placids (Figs. 2, 3B and C and 4D–H). Ring 01 has 10 primary spinoscalids consisting of a short basal part and a long end piece with a distal, curved tip (Fig. 4A and B). The basal sheath shows two long, lateral thread-like projections, overlapped by a free fringe of up to five long and flexible fringe tips. Sometimes this fringe appears bent, making the smooth basal part visible (Fig. 4B). The distal piece of the primary spinoscalids is laterally compressed and bears a fringe composed by 3–4 long fringe tips. All primary spinoscalids end in a blunt tip that appears soft in SEM. Ring 02 is composed by 10 laterally compressed spinoscalids, all formed by a long smooth basal part with a short, distal fringe. Ring 03 with 20 spinoscalids all laterally compressed with well-developed basal sheaths; these extend half the length of the spinoscalid and show a conspicuous proximal flexible spine and a distal short fringe (Fig. 4D and E). Rings 04 and 05 consist of 10 and 20 spinoscalids respectively; all resemble those of ring 03 but instead a spine they show a conspicuous flap that terminates into two fringed enlarged parts

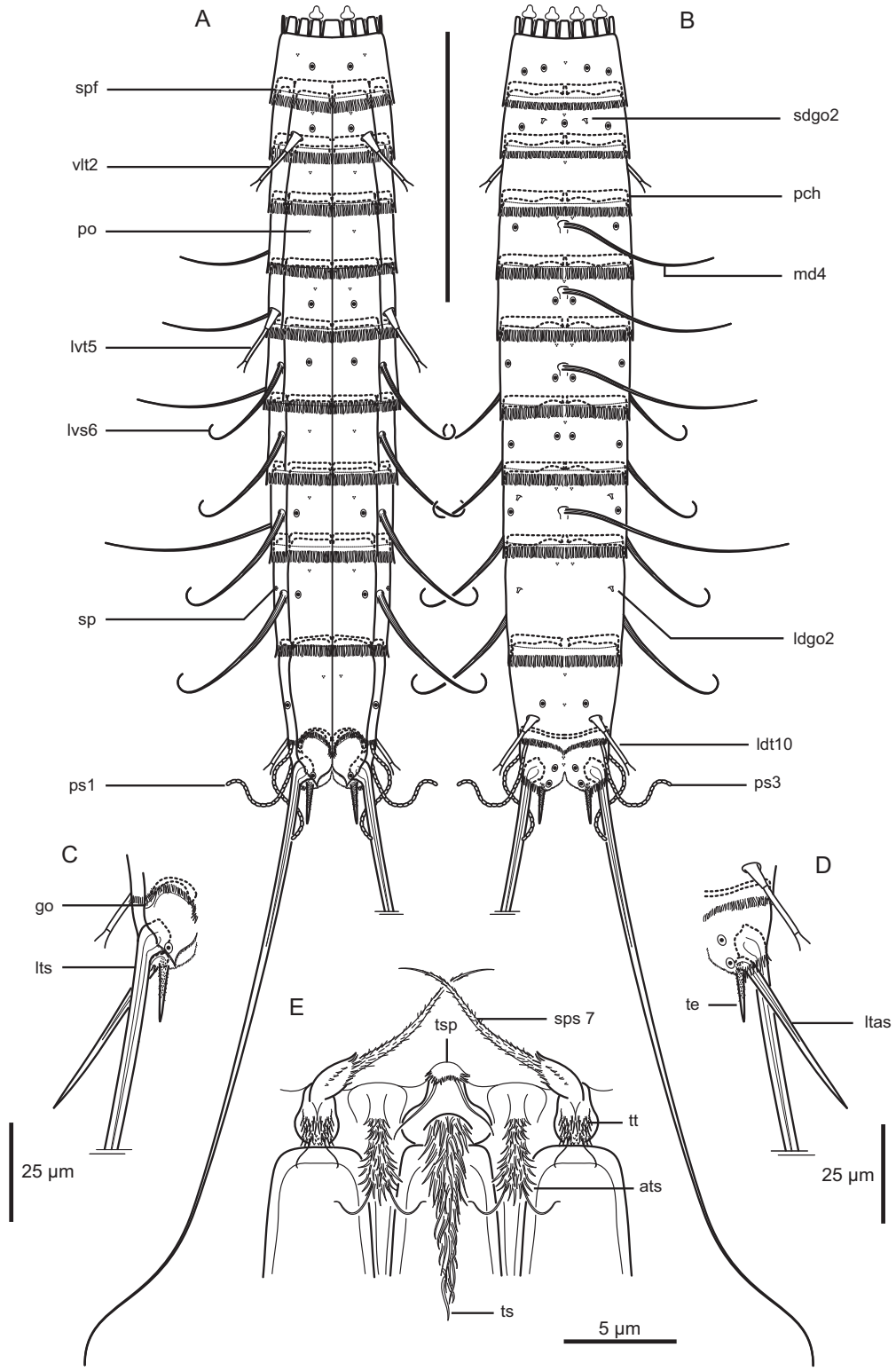
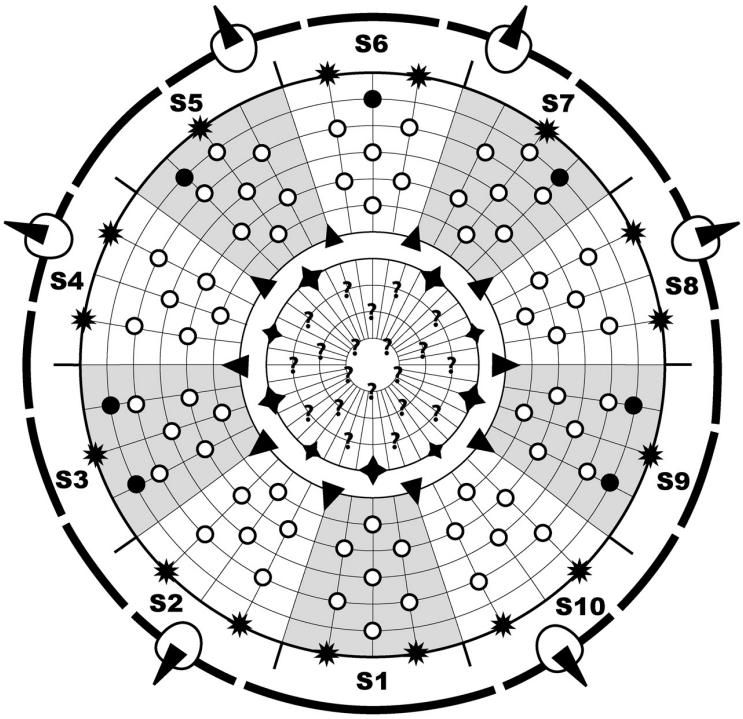


Fig. 1. *Fissuroderes sorenseni* sp. nov. line art illustration. (A) Male ventral view. (B) Male dorsal view. (C) Female ventral detail segments 10 and 11. (D) Female dorsal detail segments 10 and 11. (E) Detail of dorsal placids. Digits refer to the segment number. Scale bar A and B: 100 µm. *Abbreviations*: ats, accessory trichoscalid; go, gonopore; ldgo2, laterodorsal glandular cell outlet type 2; ldt, laterodorsal tubule; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; pch, pachycyclus; po, pore field; ps1–3, penile spines; sdgo2, subdorsal glandular cell outlet type 2; sp, sieve plate; spf, secondary pectinate fringe; sps 7, spinoscalid from seventh row; te, tergal extension; ts, trichoscalid; tsp, trichoscalid plate; tt, twin tuft; vlt, ventrolateral tubule.



Scalid and style arrangement

Ring/Section	1	2	3	4	5	6	7	8	9	10	Total
00 oos ✦	1	1	1	1	1	0	1	1	1	1	9
01 psp ▼	1	1	1	1	1	1	1	1	1	1	10
02 sp ○	1	1	1	1	1	1	1	1	1	1	10
03 sp ○	2	2	2	2	2	2	2	2	2	2	20
04 sp ○	1	1	1	1	1	1	1	1	1	1	10
05 sp ○	2	2	2	2	2	2	2	2	2	2	20
06 sp ○●	1	0	2	0	2	1	2	0	2	0	10
07 ats ✸	2	2	1	2	1	2	1	2	1	2	16
08 ts	0	1	0	1	1	0	1	1	0	1	6
Total scalids	9	9	9	9	10	9	10	9	9	9	111

Fig. 2. Diagram of mouth cone introvert and placids showing distribution of oral styles and scalids in *Fissuroderes sorenseni* sp. nov. "Double diamonds" are marked in the table with double lines and quincunxes are marked with dotted lines. Black circles symbolize spinoscalids with double fringed tufts at their bases. *Abbreviations*: ats, accessory trichoscalid; oos, outer oral style; psp, primary spinoscalid; sp, spinoscalid; ts, trichoscalid.

above the sheath (Fig. 4D and E). Ring 06 with 10 spinoscalids with the same appearance that those from rings 04 and 05 but with a shorter distal part (Fig. 4D). Ring 07 composed of 16 "accessory trichoscalids" with the same appearance as trichoscalids (see below) but shorter and not attaching through a trichoscalid plate. Unlike trichoscalids, accessory trichoscalids are always situated in between placids flanking the trichoscalids except in sector 1, 3 and 9 (Figs. 1E, 2, 3B and C and 4F–H). Additionally, ring 07 shows 7 small twin tufts underneath the spinoscalids of ring 06 on sectors 3, 5, 6, 7 and 9; a location always alternating with accessory trichoscalids referred to above (Figs. 1E, 2 and 4H). The introvert can also be described as divided into 10 sectors defined by radii drawn through spinoscalids, so each sector is delimited by two consecutive primary spinoscalids (Fig. 2). The midventral sector is numbered as 1, followed clockwise by sectors 2–10. Even sectors 2, 4, 8, 10 contain 6 spinoscalids; uneven sectors 3, 5, 7, 9 contain 8 while sectors 1 and 6 contain 7 spinoscalids. Spinoscalids are arranged as double diamonds in sectors 1, 5, 6, 7 and quincunxes in sectors 2, 3, 4, 8, 9, 10. All sectors contain 2 accessory trichoscalids except for uneven sectors 3, 5, 7 and 9. See Fig. 2 for a complete summary of oral styles, scalids and placid locations.

Six long and hairy trichoscalids attaching to small trichoscalid plates are situated in sectors 2, 4, 5, 7, 8 and 10. Trichoscalid plates are small (5 μm in width) with triangular shape and rounded edges (Figs. 1, 2, 3B and C and 4G), and articulate anteriorly with a conspicuous flap divided into two pointed fringed parts (Figs. 3B and C and 4F and G).

The neck consists of 16 trapezoid placids numbered clockwise from the midventral placid 1. All placids measure 11 μm in length and 8 μm in width at bases, except middorsal one being narrower (6 μm), and midventral one being wider (12 μm) (Figs. 1, 3B and C and 4E and G). The six trichoscalid plates are situated on ventral placids 2 and 16 and on dorsal placids 6, 8, 10, 12 (Figs. 2, 3B and C and 4E and G).

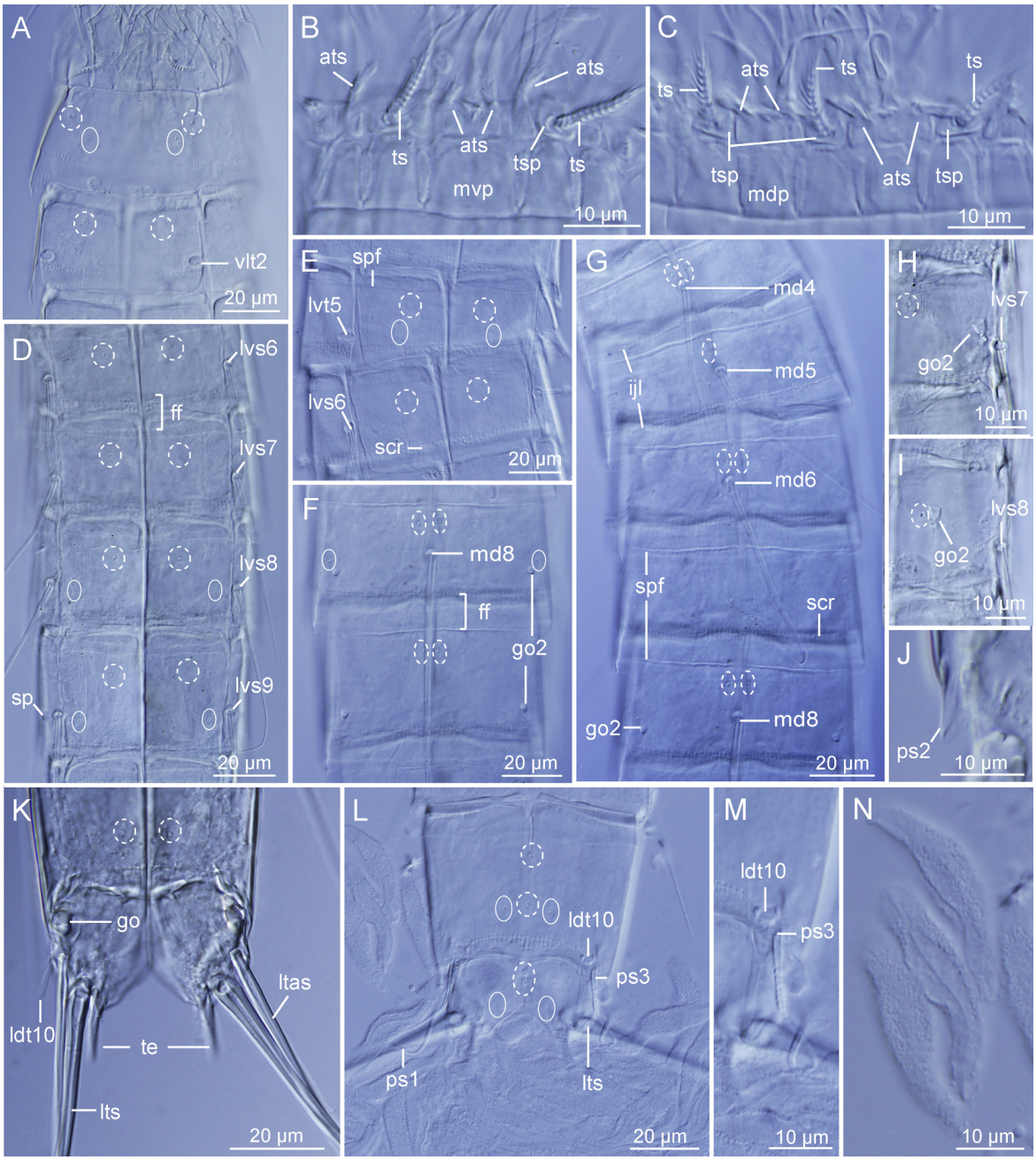


Fig. 3. Light micrographs showing details in neck and trunk morphology of *Fissuroderes sorenseni* sp. nov. (A) Paratypic male (KIN-697), detail segments 1 and 2 ventral view. (B) Paratypic male (KIN-696), detail placids, trichoscalids and accessory trichoscalids, ventral view. (C) Paratypic male (KIN-697), detail placids, trichoscalids and accessory trichoscalids, dorsal view. (D) Holotypic male, segments 6–9 ventral view. (E) Paratypic male (KIN-697), segments 5 and 6, ventral view. (F) Holotypic male, segments 8 and 9, dorsal view. (G) Holotypic male, segments 4–8, dorsal view. (H) Allotypic female, left half of segment 7, ventral view showing a glandular cell outlet type 2, dimorphic character. Compare with figure D (holotypic male) where these glandular openings are absent. (I) Allotypic female left half of segment 8, ventral view showing a glandular cell outlet type 2, dimorphic character. Compare with figure D (holotypic male) where these glandular openings are absent. (J) Paratypic male (KIN-697), detail of median penile spine (ps2) on segment 11, ventral view. (K) Allotypic female, segments 10 and 11, ventral view. (L) Paratypic male (KIN-696), segments 10 and 11, dorsal view. (M) Paratypic male (KIN-696), detail laterodorsal area segment 11, dorsal view. (N) Paratypic male (KIN-696), detail of fusiform packages (putatively immature sperm). *Abbreviations*: ats, accessory trichoscalid; ff, free flap; go, gonopore; go2, glandular cell outlet type 2; ijl, intersegmentary joint line; ldt, laterodorsal tubule; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; mdp, middorsal placid; mvp, midventral placid; ps1–3, penile spines; scr, subcuticular ornamentation; sp, sieve plate; spf, secondary pectinate fringe; te, tergal extension; ts, trichoscalid; tsp, trichoscalid plate; vlt, ventrolateral tubule. White circles indicate pore fields (dotted line) and sensory spots (solid line). Digits after abbreviations refer to segment number.

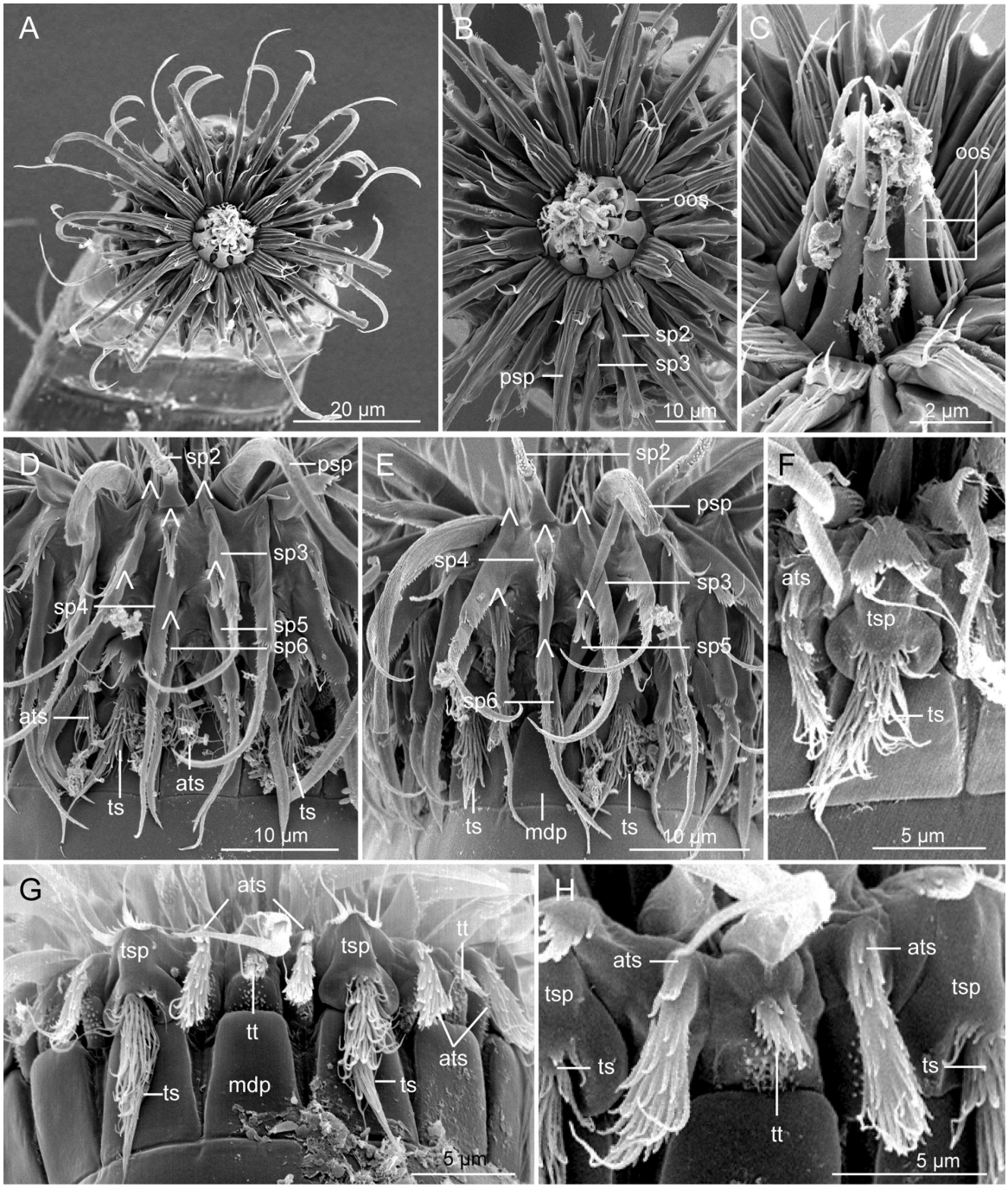


Fig. 4. Scanning electron micrographs showing introvert and mouth cone morphology in *Fissuroderes sorenseni* sp. nov. (A) Apical view of the mouth cone and introvert, note the triangular shape of the body. (B) Close up of the mouth cone and primary spinoscalids of the introvert. (C) Detail of outer oral styles of mouth cone. (D) Introvert showing sector 5. (E) Introvert showing sector 6. (F) Detail of a trichoscalid from sector 4. (G) Detail of dorsal placids, trichoscalids and seventh row of the introvert, middorsal position sectors 5–7. (H) Detail of accessory trichoscalids and twin tufts, sector 5. *Abbreviations*: ats, accessory trichoscalid; mdp, middorsal placid; oos, outer oral styles; psp, primary spinodcalids; sp 1–7, spinoscalids number refers to the row; ts, trichoscalid; tsp, trichoscalid plate; tt, twin tuft. Lambda symbols (Λ) mark attachment points of spinoscalids.

The trunk is divided into 11 segments, with only the first one consisting of a cuticular closed ring. Segments 2–11 are composed by one tergal and two sternal plates (Figs. 1A and B, 3A and 5B). The trunk is triangular in cross section (Fig. 4A).

Pore fields (glandular cell outlets type 1) situated at the anterior part of the segment, usually hidden under the pectinate fringe of the previous segment. They are very conspicuous and consist of 1–3 big pores surrounded by several small ones (Fig. 5F). Ventral

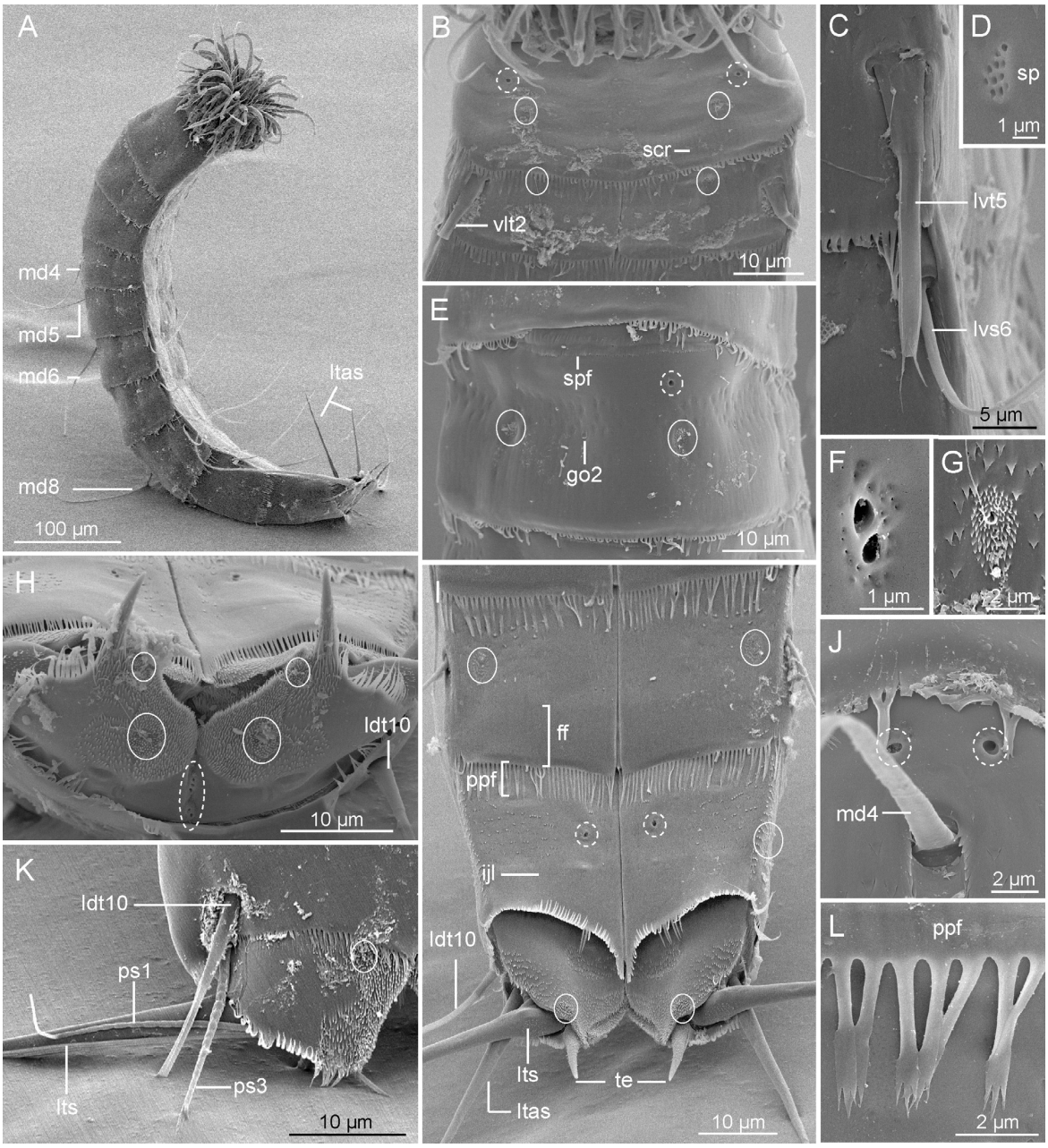


Fig. 5. Scanning electron micrographs showing trunk morphology and cuticular details in *Fissuroderes sorenseni* sp. nov. (A) Female, lateral overview. (B) Segments 1 and 2, ventral view. (C) Detail lateroventral tubule from segment 5. (D) Detail of the sieve plate, segment 9. (E) Segment 2, dorsal view. (F) Detail of a pore field, segment 10, ventral view. (G) Detail of a sensory spot segment 7, dorsal view. Note the special triangular and scale-like cuticular hairs (H) Female segment 11, dorsal view. (I) Female segments 9–11, ventral view. (J) Detail segment 4, dorsal view. (K) Male, detail of penile spines segment 11, dorsal view. (L) Detail of trifurcated primary pectinate fringe. *Abbreviations*: ff, free flap; go2, glandular cell outlet type 2; ijl, intersegmentary joint line; ldt, laterodorsal tubule; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; ppf, primary pectinate fringe; ps1–3, penile spines; scr, subcuticular relief; sp, sieve plate; spf, secondary pectinate fringe; te, tergal extension; vlt, ventrolateral tubule. White circles indicate pore fields (dotted line) and sensory spots (solid line). Digits after abbreviations refer to segment number.

pore fields all paired, situated in ventrolateral position on segment 1 and ventromedial position on segments 2–10; no pore fields are present on segment 11 (Figs. 1A, 3A, D, E and K and 5B and I). Dorsal pore fields are unpaired on segments 1, 2, 3, 5 and 7 all situated in a middorsal position; paired pore fields are present paradorsally on segments 4, 6, 8, 9 and middorsally on segment 10–11 situated one above each other (Figs. 1B, 3F, G and L and 5E, J and H).

Primary pectinate fringe well developed on all segments showing straight, elongated and flexible fringe tips, longer on the dorsal side. All fringe tips with distal tripartition (Fig. 5L).

Table 1
Measurements (in μm) of adult *Fissuroderes sorenseni* sp. nov. *Abbreviations*: LDT, laterodorsal tubule; LTAS, lateral terminal accessory spine; LTS, lateral terminal spine; LVS, lateroventral spine; LVT, lateroventral tubule; MD, middorsal spine; MSW, maximum sternal width; SW, standard width; S1–S11, segment lengths of trunk segments 1–11; TL, trunk length; VLT, ventrolateral tubule. Holotypic male in boldface. Dash – indicates that the structure could not be measured. Numbers, where inserted, indicates segment number.

Character	Holotype ♂	Allotype ♀	Paratype (KIN-696) ♂	Paratype (KIN-697) ♂
TL	**362**	349	383	399
MSW (8)	**58**	57	57	54
MSW/TL (%)	**16**	16	15	14
SW	**52**	47	56	53
SW/TL (%)	**14**	13	15	13
S1	**36**	42	39	41
S2	**38**	36	39	40
S3	**34**	34	38	37
S4	**41**	36	40	38
S5	**45**	38	41	41
S6	**45**	42	41	43
S7	**47**	44	44	46
S8	**53**	44	46	52
S9	**52**	54	51	55
S10	**59**	53	56	56
S11	**43**	43	42	43
MD4	**64**	67	–	64
MD5	**91**	89	76	82
MD6	**100**	94	94	–
MD8	**135**	–	166	–
VLT2	**–**	25	24	
LVT5	**–**	28	25	–
LVS6	**55**	47	46	47
LVS7	**65**	63	58	61
LVS8	**67**	63	59	–
LVS9	**75**	79	68	65
LDT10	**24**	24	–	–
LTS11	**228**	–	230	220
LTAS11	**–**	63	–	–

Free flap wide in all segments showing an intracuticular striated pattern, visible with both LM and SEM (Figs. 3E–G and 5B).

Secondary pectinate fringe absent on segment 1. Secondary fringes of segments 2–10 similar, consisting of one single band of minute teeth located at the edge of a short flap on each segment and predominantly hidden under primary pectinate fringe of previous segment (Fig. 5E). These secondary pectinate fringes are visible with LM forming a distinct line that can be easily confused with the *ij*-line (Fig. 3G).

Table 2
Summary of nature and location of sensory spots, glandular cells, spines and tubules arranged by series in *Fissuroderes sorenseni* sp. nov. *Abbreviations*: ac, acicular spine; go2, glandular cell outlet type 2; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; ML, midlateral; PD, paradorsal; po, pore field (glandular opening type 1); SD, subdorsal; SL, sublateral; sp, sieve plate; ss, sensory spot; tu, tubule; VL, ventrolateral; VM, ventromedial. ♀ Female condition of sexually dimorphic character.

Segment	MD	PD	SD	LD	SL	LA	LV	VL	VM
1	po		ss	ss				po	ss
2	po, ss		go2	ss				tu	po, ss
3	po								po
4	ac	po		ss					po
5	ac, po	ss					tu		po, ss
6	ac	po, ss		ss			ac		po, ss
7	po	ss		ss			ac	go2(♀)	po
8	ac	po		ss, go2			ac	ss	po, go2(♀)
9		po		go2	sp		ac	ss	po
10	po		ss	tu			ss		po
11	po, po		ss, ss			ltas (♀)	lts		ss

Segment 1 forming one complete cuticular ring (Figs. 1A, 3A and 5B). Three pairs of rounded sensory spots consisting of numerous short papillae and one cilium are located in subdorsal, laterodorsal and ventromedial positions (Figs. 1A and B, 3A and 5B). No cuticular hairs are present giving the segment a smooth appearance (Fig. 5A and B).

Segment 2 and all the remaining segments consist of one tergal and two sternal plates (Figs. 1A and 3A). One pair of wide and flexible ventrolateral tubules is present, consisting of a broad basal part and a slender distal one; this latter with lateral wing-like projections that stand out free from the tubule tip as a pair of long bristles; a round opening is visible at the end of the distal part (Figs. 1A, 3A and 5B). A pair of glandular cell outlets type 2 present in subdorsal position (Figs. 1B and 5E). Paired sensory spots in laterodorsal and ventromedial positions, plus an unpaired middorsal sensory spot (Figs. 1A and B, 3A and 5B and E). Cuticular hairs without perforation sites, triangular, short and scale-like. They form a median belt with 3 rows around the tergal plate while arranged as a ventrolateral/ventromedial patch with 4 rows on the sternal plates (Fig. 5E). An additional cluster with minute hairs is located paraventrally on this and the following segments.

Segment 3 without sensory spots, tubules or spines. Hair pattern as described on previous segment (Fig. 1A and B).

Segment 4 with a long, flexible and distally flattened middorsal spine arising from a cuticular, minutely fringed groove (similar on segments 5, 6 and 8) (Figs. 1B, 3G and 5A and J). A pair of laterodorsal rounded sensory spots is present. Cuticular hairs forming subdorsal and lateroventral clusters. Paradorsal patch of minute hairs barely seen even with SEM surrounding the middorsal spine (Fig. 5J). Sternal hair pattern and pectinate fringe as described on previous segments.

Segment 5 with a long, flexible and distally flattened middorsal spine as on segment 4 (Figs. 1B, 3G and 5A). One pair of wide lateroventral tubules with the same appearance as those described from segment 2 (Figs. 1A, 3E and 5C). Paired big oval sensory spots in paradorsal and ventromedial positions (Figs. 1A and B and 3E). Cuticular hair pattern as described on previous segments.

Segment 6 with a middorsal spine as described on segments 4 and 5 and a pair of long, thin, flexible and slightly flattened lateroventral spines (Figs. 1A and B, 3D and G and 5A and C). Three pairs of oval sensory spots present in paradorsal, laterodorsal and ventromedial positions (Fig. 1A and B). Paradorsal sensory spots slightly larger than the others, ventromedial sensory spots much smaller than those on previous segment. Cuticular hair pattern as described on preceding segments.

Segment 7 without middorsal spine (Figs. 1B, 3G and 5A). A pair of long and flexible lateroventral spines present as on segment 6 (Figs. 1A and 3D). A pair of glandular cell outlets type 2 is present in ventrolateral position in females (Fig. 3H) but not in males (Figs. 1A and 3D). A pair of oval sensory spots present in paradorsal positions, and a pair of smaller ones in laterodorsal positions. Cuticular hair pattern as described on previous segments.

Segment 8 with a long, flexible and distally flattened middorsal spine as on segments 4, 5 and 6, and a pair of long lateroventral spines as described on segments 6 and 7 (Figs. 1A and B, 3D, F and G and 5A). Two pairs of glandular cell outlets type 2 are present in laterodorsal (Figs. 1B and 3F and G) and ventromedial positions; the latter is only present in females (Fig. 3I) and absent in males (Figs. 1B and 3D). Two pairs of oval sensory spots located in laterodorsal and ventrolateral positions (Figs. 1A and B and 3D and F). Ventrolateral sensory spots as small as those on segment 6. Cuticular hair pattern as described on previous segments.

Segment 9 with a pair of long and flexible lateroventral spines as described on segments 6–8 (Figs. 1A and 3D). One pair of small glandular cell outlets type 2 present in laterodorsal position

(Figs. 1B and 3F). One pair of big oval sensory spots in ventrolateral positions located at the same level as the lateroventral spines (Figs. 1A and 5I). Paired small sieve plates composed of 11–12 pores are present in sublateral positions (Figs. 3D and 5D). Cuticular hair pattern as on previous segments with an additional band of long and thin hairs without perforation sites located along the tergosternal articulation (Fig. 5I).

Segment 10 without spines (Figs. 1A and B, 3K and L and 5I). A pair of laterodorsal very long and flexible tubules are situated close to the posterior margin of the segment, with the same appearance as those on segments 2 and 5 (Figs. 1B, 3K–M and 5H, I and K). Paired oval sensory spots situated in subdorsal and lateroventral positions (Figs. 1A and B, 3L and 5I). Posterior margin of the segment with a paraventral extension reaching the posterior margin of segment 11 midventrally (Figs. 1A and 5I). The pectinate fringe is very short ventrolaterally, but increasing in length toward the midventral area (Fig. 5I). Cuticular hairs arranged in ventromedial/ventrolateral wide clusters on the sternal plates (Fig. 5I) and in two subdorsal and laterodorsal clusters on tergal plates. An additional band of longer and thinner hairs along the tergosternal junction as described on segment 9 (Fig. 5I). Females with a pair of ventrolateral sclerotized and oval gonopores situated in the anterior part of the segment and usually overlapped by the free flap of segment 9 (Figs. 1C and 3K).

Segment 11 with one pair of long and flexible lateral terminal spines (Figs. 1A–D, 3K and L and 5I and K). Females with a pair of shorter and strong lateral terminal accessory spines (Figs. 1C and D, 3K and 5A and I), males with three pairs of penile spines instead (Figs. 1A and B, 3L and M and 5K). First and third pairs of penile spines (p1, p3) are long, flexible and crenulated while the second pair (p2) is short, conical and stout, and easily mistaken with the border of segment 11 that forms a lateral spinose process (Figs. 1A and B, 3L, M and J and 5K). Tergal extensions are 16 μm long and spinose, projecting posteriorly and showing fringed ventrolateral margins (Figs. 1B and 5H and I). Sternal plates rounded with slightly pointed extensions in the ventromedial area, showing fringed margins in ventrolateral positions close to the insertion of the lateral terminal spines (Figs. 1A and C and 5I). Two paired large and oval sensory spots present in subdorsal positions (Figs. 1B and D, 3L and 5H and K) plus an additional ventromedial pair close to the sternal extensions (Figs. 1A and C and 5I). Cuticular short and filiform hairs without perforation sites covering subdorsal and ventromedial areas as well as the posterior margins of tergal and sternal plates (Fig. 5H, K and I).

When one of the paratypes (paratype 1) was mounted for LM, the pressure of the coverslip broke off a part of the last segment, letting a large amount of fusiform packages escape from the trunk (Fig. 3L and N). These packages measure around 20 μm in length showing a granulated aspect and a well-defined longitudinal line in the middle (Fig. 3N). They have been putatively identified as sperm clusters.

3.1.6. Remarks

The combination of segment 1 forming a closed ring, aligned middorsal spines, presence of lateral terminal spines and absence of midterminal spine assigns the new species to Echinoderidae. Within Echinoderidae, the main diagnostic character to differentiate genera relates to the composition of the second segment. Segment 2 in *Echinoderes* is composed of a closed ring; of one tergal plate with incomplete tergosternal divisions in *Meristoderes*; of one tergal plus one sternal plate in *Cephalorhyncha* Adrianov, 1999 in Adrianov and Malakhov, 1999; and one tergal plus two sternal plates in *Fissuroderes* and *Polacanthoderes*. *F. sorenseni* sp. nov. is easily distinguished from species of *Echinoderes*, *Cephalorhyncha* and *Meristoderes* by the presence of completely differentiated sternal plates on segment 2 (see Herranz et al., 2012). Although *Fissuroderes* and *Polacanthoderes* share the composition of the second segment, they differ in the presence of serial spines situated in unusual positions, characteristic for *Polacanthoderes* (Sørensen, 2008a) and absent in *Fissuroderes*. The absence of these serial spines in the new species places it clearly inside the genus *Fissuroderes* (but see below, Morphological characters in *Fissuroderes*).

Among species of *Fissuroderes*, *F. sorenseni* sp. nov. is easily recognized by its unique middorsal spine pattern, with four middorsal spines on segments 4, 5, 6 and 8, with a remarkable lack in segment 7; the remaining species in the genus show five middorsal spines on segments 4–8 (Neuhaus and Blasche, 2006). *F. sorenseni* sp. nov. also differs from other *Fissuroderes* species by the presence of subdorsal glandular cell outlets type 2 on segments 2, 7, 8 and 9. The presence of glandular cell outlets type 2, the tubule pattern, the middorsal and lateroventral spine pattern and the tergal extensions appearance seem to be the key characters to differentiate species within the genus. Therefore *F. higginsi* is distinguished by the presence of laterodorsal tubules on segment 2, sublateral tubules on segment 8 and the absence of a ventrolateral spine on segment 8. *F. novaezealandia* is discriminated by the presence of subdorsal glandular openings type 2 on segment 2 and the divided tergal plate of segment 11 with long tergal extensions. *F. papai* is differentiated by the presence of lateral accessory tubules on segment 8 and a divided 11th tergal plate with short tergal extensions. *F. rangi* is distinguished by the absence of tubules on segments 2 and 5, and the presence of non-divided tergal plate of segment 11 with very long tergal extensions. *F. thermoi* is differentiated by the presence of sublateral tubules on segment 10 and divided tergal plates on segment 11with short and rounded tergal extensions.

Below follows a dichotomous short key to ease identification of species of *Fissuroderes* based on the characters mentioned above. The presence of a division in the tergal plate on segment 11 was not used as a character to build this key because it is very difficult to see, as it is usually hidden under the posterior margin of segment 10.

3.1.6.1. Dichotomous identification key to species of Fissuroderes.

1.	Middorsal spines present on segments 4–8	2
	Middorsal spines present on segments 4, 5, 6 and 8, elongated and pointed tergal extensions, ventrolateral wide tubules on segment 2, lateroventral on segment 5 and laterodorsal on segment 10	*F. sorenseni* sp. nov.
2.	Presence of glandular cell outlets type 2	3
	Absence of glandular cell outlets type 2, lateral accessory tubules present on segment 8, short and pointed tergal extensions	*F. papai*
3.	Four or more pairs of glandular cell outlets type 2	4
	Two pairs of glandular cell outlets type 2 in subdorsal positions on segments 6 and 9, extraordinary long, needle-shaped tergal extensions	*F. rangi*
4.	Paired glandular cell outlets type 2 in laterodorsal position on segments 5, 6, 8 and 9	5
	Paired glandular cell outlets type 2 in laterodorsal position on segments 5, 6, 8 and 9 plus an additional pair in subdorsal position on segment 2	*F. novaezealandia*
5.	Short and rounded tergal extensions, lateroventral spines on segments 6 to 9, ventrolateral tubules on segment 2 and lateroventral on segment 5	*F. thermoi*
	Long and pointed tergal extensions, lateroventral spines on segments 6, 7, 9, ventrolateral and laterodorsal tubules on segment 2 and lateroventral on segment 5	*F. higginsi*

3.1.6.2. Morphological characters in Fissuroderes.

Fissuroderes was the third genus assigned to Echinoderidae (Neuhaus and Blasche, 2006) after *Cephalorhyncha* (Adrianov, 1999 in Adrianov and Malakhov, 1999) and *Echinoderes* (Claparéde, 1863). Initially, these genera were very clearly differentiated by the composition of segment 2, but with the description of the genus *Polacanthoderes* inside this family some of the diagnostic characters of *Fissuroderes*, such as

the composition of segment 2, were shared and then not exclusive. Therefore, the only exclusive and positive diagnostic character of *Fissuroderes* was the presence of a pair of sexually dimorphic ventral gland cell outlets type 2 in females. As pointed out by Sørensen (2008b) an exploration for new reliable and consistent characters is needed for this genus, which has never been reported again after its original description.

The use of combined SEM and LM in the description of *F. sorenseni* sp. nov. allowed us to find several additional new characters that somehow could be used to complete the diagnosis of the genus. Unfortunately, most of these characters were not reported before for any of the previously described species of *Fissuroderes*. However, this fact does not mean that all these characters are not present in the described species; it is possible that some of them might not have been detected knowing that the original descriptions were based on LM only.

3.1.6.2.1. Introvert. The mouth cone shows nine outer oral styles alternating in length but not in size. Differences between long and short styles are not as conspicuous as in species of *Dracoderes*, *Paracentrophyes* and *Neocentrophyes* (where the short styles are several times smaller) but still evident in the length of the basal parts. This is the first report of an echinoderid species displaying this character, however the authors have detected minor size differences among other species belonging to the genera *Echinoderes* and *Meristoderes*. A revision of this character in other echinoderid genera and species would be desirable in order to evaluate its phylogenetic relevance.

The spinoscalid composition of the first five rings of the introvert does not differ from the common pattern shared by most cyclorhagids, with rings 06 and 07 being the most variable ones and showing multiple organizations. In *F. sorenseni* sp. nov. ring 06 contains 10 spinoscalids and ring 07 is composed of 16 accessory trichoscalids always flanking the trichoscalids and additionally present as pairs in sector 1 and singles in sectors 3and 9. The presence of special types of appendages in the last rows of the introvert was described for the first time in species of *Meristoderes*, the so called leaf-like scalids (Sørensen et al., 2013). Similar appendages were illustrated but not described by Nebelsick (1993) in *Echinoderes capitatus* Zelinka, 1928 and found posteriorly in additional species of *Echinoderes* (Herranz, pers. obs.). The "accessory trichoscalids" constitute a new variation of the introvert appendages not reported for any of the known species of the phylum. The general appearance resembles a reduced trichoscalid but without an associated trichoscalid plate. Surprisingly, the appearance of the "accessory trichoscalids" is very similar to the homalorhagid trichoscalids, except for the number (14 in homalorhagids vs. 16 in the new species) and for the additional presence of "regular" trichoscalid with plates in *F. sorenseni* sp. nov. lacking in homalorhagid species (see Brown, 1989; Neuhaus, 2012; Sánchez et al., 2014). Moreover, and to further complicate things, the small twin tufts alternating with the accessory trichoscalids (see introvert description) have the same appearance but are much shorter and reduced, and could hence be interpreted as rudimentary accessory trichoscalids. Further comparative studies are needed to clarify and evaluate the significance of trichoscalid characters in the whole phylum. Studying and mapping the last rows of introvert appendages is a difficult task because these scalids usually are hidden under the scalids from previous rings and therefore difficult to see. Unfortunately, no introvert data are available from the remaining species of *Fissuroderes*, consequently we cannot determine whether the presence of accessory trichoscalids and twin tufts are species or genus specific.

Generally in Kinorhyncha, the spinoscalids share a similar morphology, having a proximal part articulated with a distal part that shows variations in the length, getting gradually shorter and more hairy toward the last rings of the introvert. Functionally, the distribution of the primary spinoscalids (used to define sectors in the introvert) influences the position of all remaining spinoscalids, so the radial position occupied by the primary spinoscalids is free in all remaining rows. This arrangement clearly saves space for the primary spinoscalids once the introvert is withdrawn. The same influence continues in the posterior appendages of the introvert that, still influenced by the spinoscalid position, also seem to depend on the position of the trichoscalids attached to trichoscalid plates, in turn intimately associated with the neck. Quite often, single spinoscalids may be "missing" in posterior rings of certain sectors, and this lack is usually due to the presence of a trichoscalid that takes up the space for the spinoscalid (Herranz et al., 2012; Sørensen et al., 2013). As a consequence, the distribution of the last spinoscalid rows does not always follow the radially symmetrical patterns found for other spinoscalids (Sørensen and Pardos, 2008). Therefore the introvert and neck are two body regions being closely linked and functionally dependent from each other, with mutual influences that make the limit between them very difficult to be established. All these observations reinforce the assumption already accepted and applied in all kinorhynchs to not consider the introvert and neck as true segments (Neuhaus and Higgins, 2002; Sørensen and Pardos, 2008).

3.1.6.2.2. Trunk. The presence of pore fields consisting of 2–3 conspicuous big holes is very remarkable in the trunk. It is known that this trait may vary during the life of the animal and among adult specimens (Gª Ordóñez et al., 2000), hence the presence of these glandular openings should be used with caution as a taxonomic feature. However, the same appearance in all studied specimens would be indicative of a similar age of the animals.

The morphology of the tubules in the new species fits the classical echinoderid tubule but shows features not described before for any other kinorhynch, such as the unusually enlarged base and the conspicuously extended lateral projections at the distal end.

F. sorenseni sp. nov. differs from all other species, not only within the genus but at least in the family Echinoderidae, in having markedly triangular and scale-like cuticular hairs without perforation sites (see Fig. 5G). This is in contrast to the common pattern of relatively long, filiform hairs with perforation sites. However, the presence of abundant hairs without perforation sites is a very common feature among species of *Antygomonas*, *Echinoderes*, *Centroderes*, *Campyloderes*, *Semnoderes*, *Tubulideres* and *Zelinkaderes*, although hairs in these species are usually short and filiform.

The presence of two pairs of long, flexible and crenulated penile spines plus one pair short, stout and straight ones is another character only observed in *F. sorenseni* sp. nov. The presence of three paired penile spines is a common trait shared by all the species of the genus *Fissuroderes*, however, none of these species was reported as having crenulated penile spines (Neuhaus and Blasche, 2006). A re-examination of these species could not determine with certainty the nature of this feature in all the specimens. Outside *Fissuroderes* the presence of crenulated penile spines has only been reported from one echinoderid species: *Echinoderes neospinosus* Gª Ordóñez et al., 2008 (see Gª Ordóñez et al., 2008). Outside Echinoderidae only species of *Dracoderes* have crenulated penile spines (Sørensen et al., 2012b). Species of several additional genera share the presence of sexually dimorphic crenulated spines on segment 10, usually a single middorsal one and a laterodorsal pair: *Centroderes*, *Triodontoderes*, *Tubulideres*, *Wollunquaderes* and *Zelinkaderes* (see Bauer-Nebelsick, 1995; Sørensen et al., 2007; Sørensen and Rho, 2009; Sørensen and Thormar, 2010; Neuhaus et al., 2013). Interestingly, all these genera show prominent midterminal spines. The function of the crenulated spines, whether on segment 10 or 11, is still unknown, although could probably be related with reproductive activities.

The sexual dimorphism in *F. sorenseni* sp. nov. is not restricted to the presence of penile spines in males vs. lateral terminal

accessory spines in females. Also two extra pairs of ventral glandular openings type 2 appear in segments 7 and 8 of females only. This sexually dimorphic trait is found in all species of *Fissuroderes*, and exclusively within this genus, and can therefore be considered autapomorphic.

Unfortunately and as noticed above, the descriptions of the already known species of *Fissuroderes* lack details from SEM. Consequently, features such as the appearance of the glandular openings and the structure of tubules cannot be assessed with certainty.

The fusiform packages with a granulated appearance found in one of the male specimens are putatively identified as sperm clusters. The conspicuous longitudinal midline could be interpreted as a crack point that releases the sperm when mature. Alternatively, these packages may be considered spermatophores. Spermatophores have been reported from homalorhagids and from one specimen of *Centroderes*. They are hollow spheres containing the sperm without a special organization (Brown, 1983; Neuhaus, 2012; Herranz et al., in press). The appearance of the structures in *F. sorenseni* sp. nov. do not fit with any of the spermatophore descriptions; moreover, the structures described herein are internal, only seen after accidental squeezing the specimen. Therefore we tentatively identify these packages as immature sperm clusters. Future studies through culturing methods are needed to clarify the reproductive processes in kinorhynchs.

Genus *Meristoderes* Herranz, Thormar, Benito, Sánchez and Pardos, 2012

3.2. *M. boylei sp. nov.* (*Figs. 6–10* and *Tables 3 and 4*)

3.2.1. *Diagnosis*

Meristoderes with middorsal spines on segments 4, 6 and 8, increasing uniformly in length posteriorly. Ventrolateral tubules on segment 2. One pair of lateroventral tubules on segment 5; lateroventral spines on segments 6–9; lateral accessory tubules on segment 8; one pair of laterodorsal tubules on segment 10 in males and females; very long lateral terminal spines (exceeding the trunk length) on segment 11 in both sexes and short lateral terminal accessory spines on segment 11 in females only. Tergal extensions of segment 11 elongated with pointed tips.

3.2.2. *Type material*

Holotype: adult male collected on 20 September 2012, 3 miles off Fort Pierce, Florida 27°29.73′ N, 80°13.83′ W at 11 m depth from muddy sand; mounted in Fluoromount G® and deposited at the Natural History Museum of Denmark under accession number ZMUC KIN-701. No allotype or paratypes are designated.

3.2.3. *Additional material*

One male and one female collected from the same locality as the holotype were prepared for SEM and stored in the Meiofauna Laboratory at the Universidad Complutense of Madrid.

3.2.4. *Etymology*

The new species is named after Dr. Michael J. Boyle from the Smithsonian Marine Station at Fort Pierce, in gratitude for all the help and support provided to M. Herranz during her stay in Florida.

3.2.5. *Description*

Adults with head, neck and eleven trunk segments (Figs. 6A and B, 8A andB and 9A and C). Measurements and dimensions are given in Table 3. A summary of sensory spots, spines, tubules and glandular outlets positions is provided in Table 4.

The head consists of a retractable mouth cone and an introvert with seven rings of scalids (Figs. 7 and 9A, B and D–F). Outer armature of the mouth cone formed by nine outer oral styles slightly alternating in size between 5 larger ones situated anterior to uneven sectors of the introvert, and 4 shorter ones situated anterior to even sectors; middorsal outer oral style is missing (Figs. 7 and 9E). All outer oral styles divided into two articulated subunits: a long and smooth basal part and a distal, curved and elongated part ending in a thin tip (Fig. 9B and E). Inner armature of the mouth cone could not be examined in detail.

The introvert has seven rings of spinoscalids and one additional ring of trichoscalids that are associated with the placids (Figs. 7 and 9B, D and F). Ring 1 has 10 primary spinoscalids consisting of two parts. The basal part of every primary spinoscalid is equipped with two overlapping fringes, each one with four long spines (Fig. 9B). The distal part is flattened and finger like, ending with a filiform tip. Ring 2 is formed by 10 laterally flattened spinoscalids, all formed by a basal part covered with a sheath that terminates into a fringe (Fig. 9B, D and F); distal part flat and elongated with pointed tip. Ring 3 formed by 20 scalids all with conspicuous sheaths around their proximal halves. These sheaths resemble a gable roof with a long central flexible spine (Fig. 9D and F). Rings 4 and 5 consist of 10 and 20 spinoscalids, respectively. All spinoscalids in rings 4 and 5 similar to those of ring 3 but instead the central spine they show a conspicuous flap ending in two fringed parts above the sheath (Fig. 9D and F). Ring 6 with 6

Table 3
Measurements (in μm) of adult *Meristoderes boylei* sp. nov. *Abbreviations*: LAT, lateral accessory tubule; LDT, laterodorsal tubule; LTAS, lateral terminal accessory spine; LTS, lateral terminal spine; LVS, lateroventral spine; LVT, lateroventral tubule; md, middorsal spine; MSW, maximum sternal width; SW, standard width; S1–S11, segment lengths of trunk segments 1–11; TL, trunk length; VLT, ventrolateral tubule. Numbers, where inserted, indicates segment number.

Character	Holotype ♂	Character	Holotype ♂
TL	313	MD4	63
MSW (8)	46	MD6	91
MSW/TL (%)	15	MD8	102
SW	41	VLT2	15
SW/TL (%)	13	LVT5	24
S1	33	LVS6	27
S2	30	LVS7	28
S3	28	LVS8	33
S4	27	LAT8	26
S5	29	LVS9	30
S6	34	LDT10	17
S7	34	LTS11	353
S8	39		
S9	40		
S10	40		
S11	37		

Table 4
Summary of nature and location of sensory spots, glandular cells, spines and tubules arranged by series in *Meristoderes boylei* sp. nov. *Abbreviations*: ac, acicular spine; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; ML, midlateral; PD, paradorsal; po, pore field (glandular cell outlet type 1); SD, subdorsal; SL, sublateral; sp, sieve plate; spo, single pore opening; ss, sensory spot; tu, tubule; VL, ventrolateral; VM, ventromedial. ♀ Female condition of sexually dimorphic character.

Segment	MD	PD	SD	LD	SL	LA	LV	VL	VM
1	po		ss	ss				po	ss
2	po, ss			ss				tu	po, ss
3	po		ss	ss					po
4	ac	po							po
5	po			ss			tu		po, ss
6	ac	po, ss		ss			ac		po, ss
7	po		ss	ss			ac		po, ss
8	ac	po, ss				tu	ac		po
9	spo	po, ss	ss	ss	sp		ac	ss	po
10	po		ss	tu				ss	po
11		ss	ss			ltas (♀)	lts	ss	

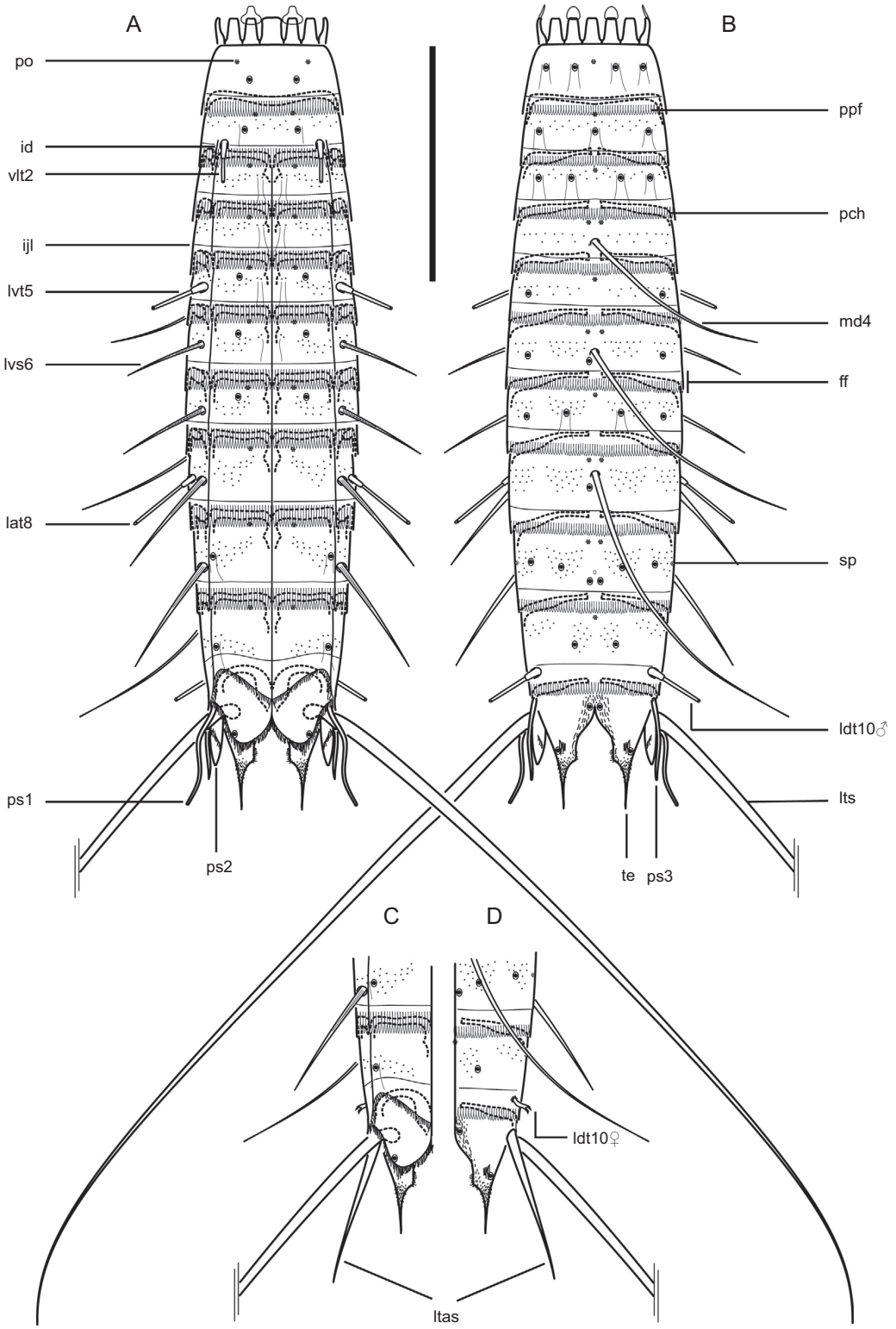
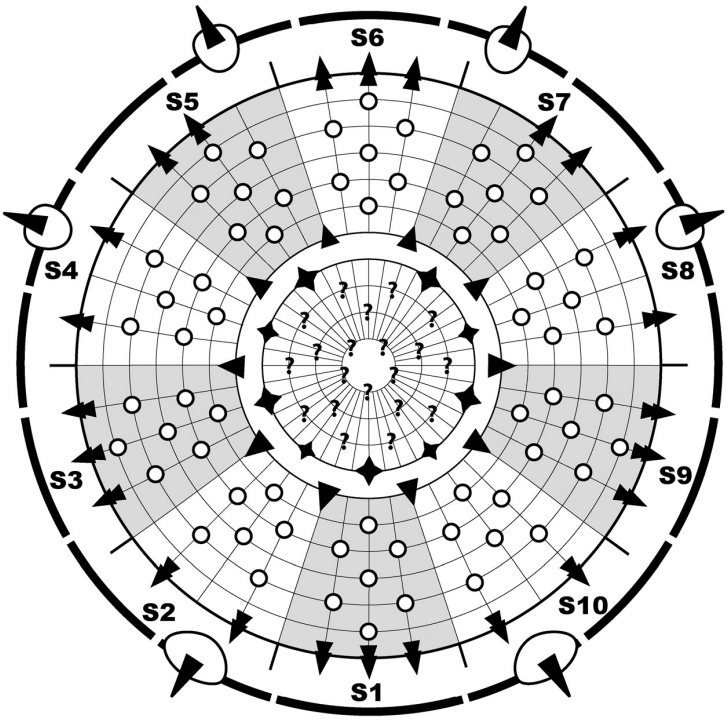


Fig. 6. *Meristoderes boylei* sp. nov. line art illustration. (A) Male ventral view. (B) Male dorsal view. (C) Female detail of segments 9–11 right side ventral view. (D) Female detail of segments 9–11 right side, dorsal view. *Abbreviations*: ff, free flap; id, incomplete division; ijl, intersegmentary joint line; lat, lateral accessory tubule; ldt, laterodorsal tubule; lts, lateral terminal spine; ltas, lateral terminal accessory spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; pch, pachycyclus; po, pore field; ppf, primary pectinate fringe; ps1–3, penile spines; sp, sieve plate; te, tergal extension; vlt, ventrolateral tubule. Digits after abbreviations refer to segment number.



Scalid and style arrangement

Ring/Section	1	2	3	4	5	6	7	8	9	10	Total
00 oos ✦	1	1	1	1	1	0	1	1	1	1	9
01 psp ▼	1	1	1	1	1	1	1	1	1	1	10
02 sp ○	1	1	1	1	1	1	1	1	1	1	10
03 sp ○	2	2	2	2	2	2	2	2	2	2	20
04 sp ○	1	1	1	1	1	1	1	1	1	1	10
05 sp ○	2	2	2	2	2	2	2	2	2	2	20
06 sp ○	1	0	1	0	1	1	1	0	1	0	6
07 ls ▼	3	2	3	2	2	3	2	2	3	2	24
08 ts ⚲	0	1	0	1	1	0	1	1	0	1	6
Total scalids	10	9	10	9	10	10	10	9	10	9	115

Fig. 7. Diagram of mouth cone, introvert and placids showing distribution of oral styles and scalids in *Meristoderes boylei* sp. nov. "Double diamonds" are marked in the table with double lines and quincunxes are marked with dotted lines. *Abbreviations*: ls, leaf-like scalid; oos, outer oral style; psp, primary spinoscalid; sp, spinoscalid; ts, trichoscalid.

spinoscalids shorter than those in preceding rings and also showing shorter sheaths. Ring 07 is formed by 24 leaf-like scalids with a wide and hairy base from where several flexible elongations arise. The introvert can also be described as divided into 10 sectors, being each sector delimited by two consecutive spinoscalids (Fig. 7). The midventral sector is numbered as 1, followed clockwise by sectors 2–10. Sectors 2, 4, 8 and 10 contain 8 spino/leaf-like scalids; sectors 5 and 7 contain 9 and sectors 1, 3, 6 and 9 contain 10. Spinoscalids are arranged as double diamonds in sectors 1, 3, 5, 6, 7, 9 and forming quincunxes in sectors 2, 4, 8, 10. See Fig. 7 for a polar diagram that summarizes the location and arrangement of oral styles, scalids and placids.

Six trichoscalids, four dorsal and two ventral, are present on their respective trichoscalid plates on sectors 2, 4, 5, 7, 8 and 10 (Figs. 7, 8C and 9D, F and G).

Neck composed of 16 placids numbered clockwise from the midventral 1 (Fig. 7). Placids 2–16 are trapezoid measuring 7 μm at the base while the midventral placid is more rectangular and wider measuring 9 μm (Figs. 6A and B, 8C and 9D and F). All placids articulate with the anterior edge of the first trunk segment. Trichoscalid plates bearing trichoscalids appear dorsally on placids 6, 8, 10, 12 and ventrally on placids 2 and 16 (Fig. 7). Dorsal trichoscalid plates are rounded and small measuring 6 μm at the base (Fig. 9G) while ventral plates are larger and triangular, with enlarged bases 8 μm wide (Fig. 8C).

The trunk is divided into 11 segments (Figs. 6A and B, 8A and B and 9A and C). Segment 1 consists of one closed cuticular ring; segment 2 formed by one closed cuticular ring with incomplete tergosternal divisions (Figs. 8C and 10B); segments 3–11 composed of one tergal and two sternal plates (Figs. 6A and B, 8A and B, 9A and C and 10A).

Rounded gland pore fields consisting in numerous minute pores (20+), situated in the anterior part of the segments above to the hair perforation sites and usually hidden under the posterior part of the previous segment (Fig. 10B and G). Dorsal pore fields are unpaired middorsally on segments 1–3, 5, 7, 10 and paired paradorsally

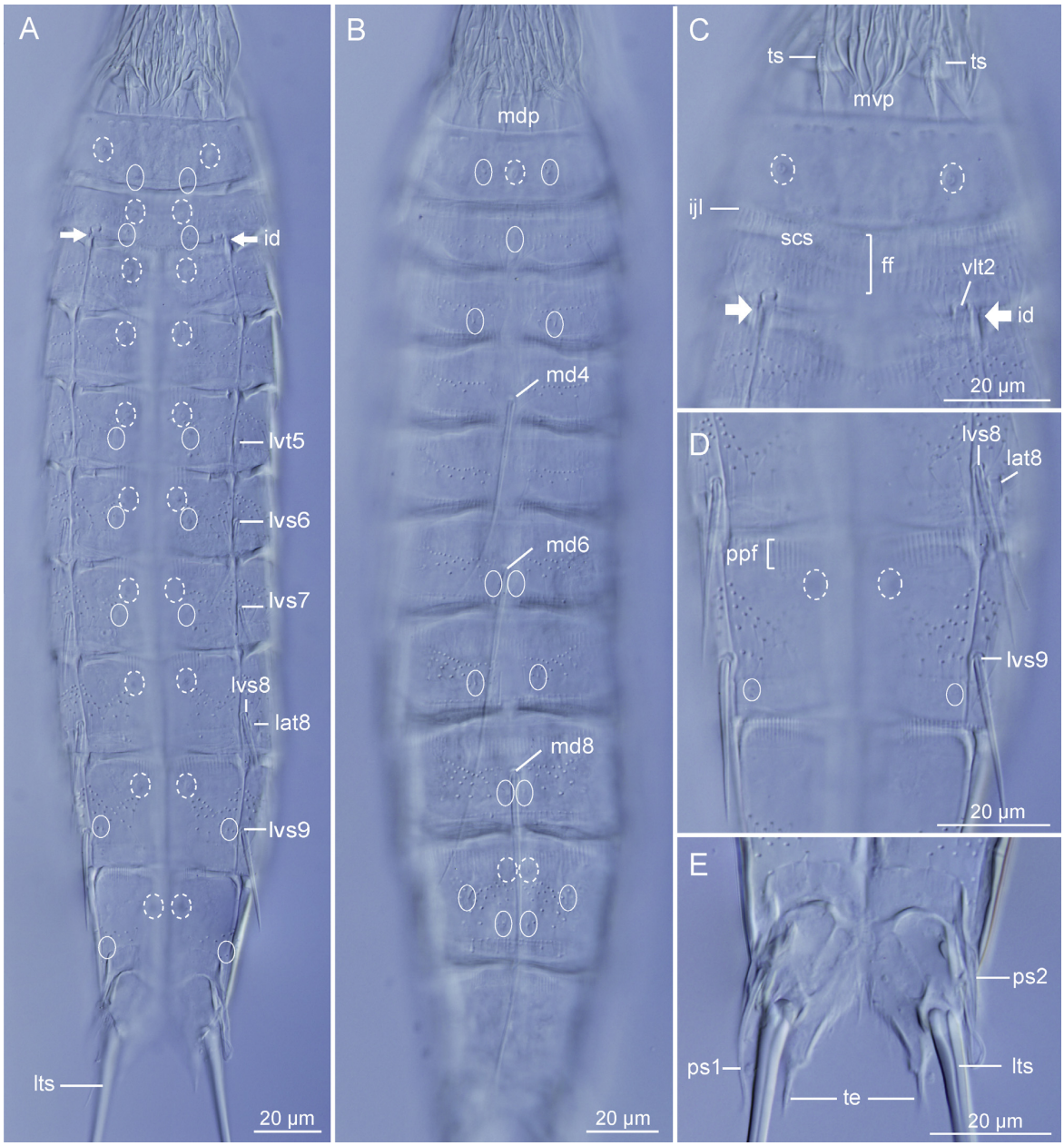


Fig. 8. Light micrographs showing details in neck and trunk morphology of *Meristoderes boylei* sp. nov. holotypic male. (A) Ventral view. (B) Dorsal view. (C) Detail of neck and segments 1–3, ventral view. (D) Detail segments 8 and 9, ventral view. (E) Segments 10 and 11, ventral view. *Abbreviations*: ff, free flap; id, incomplete division; ijl, intersegmentary joint line; lat, lateral accessory tubule; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tubule; md, middorsal spine; mdp, middorsal placid; mvp, midventral placid; ppf, primary pectinate fringe; ps1–3, penile spines; scs, subcuticular striation; te, tergal extension; vlt, ventrolateral tubule; ts, trichoscalid. White circles indicate pore fields (dotted line) and sensory spots (solid line). Digits after abbreviations refer to segment number.

on segments 4, 6, 8, 9 (Figs. 6B and 8B). A single pore opening (glandular opening type 1) is present in middorsal position on segment 9 (Fig. 10J). Ventral pore fields are present in a ventrolateral position on segment 1, and in ventromedial position on segments 2–10 (Figs. 6A and 8A); no pore fields appear on segment 11.

Primary pectinate fringe well developed in all segments showing regular, wide and flexible fringe tips with a trident shape (Fig. 10E and F).

Secondary pectinate fringe absent on segment 1. Secondary fringes of segments 2–10 similar, consisting of one single band of minute irregular teeth on each segment usually hidden under the primary pectinate fringe of previous segment (Fig. 10B and G).

Wide free flap in all segments arising below the level of sensory spots and showing intracuticular striations visible both with LM and SEM (Figs. 8C and 10B).

Segment 1 consists in one closed cuticular ring. Paired subdorsal, laterodorsal and ventromedial sensory spots are present

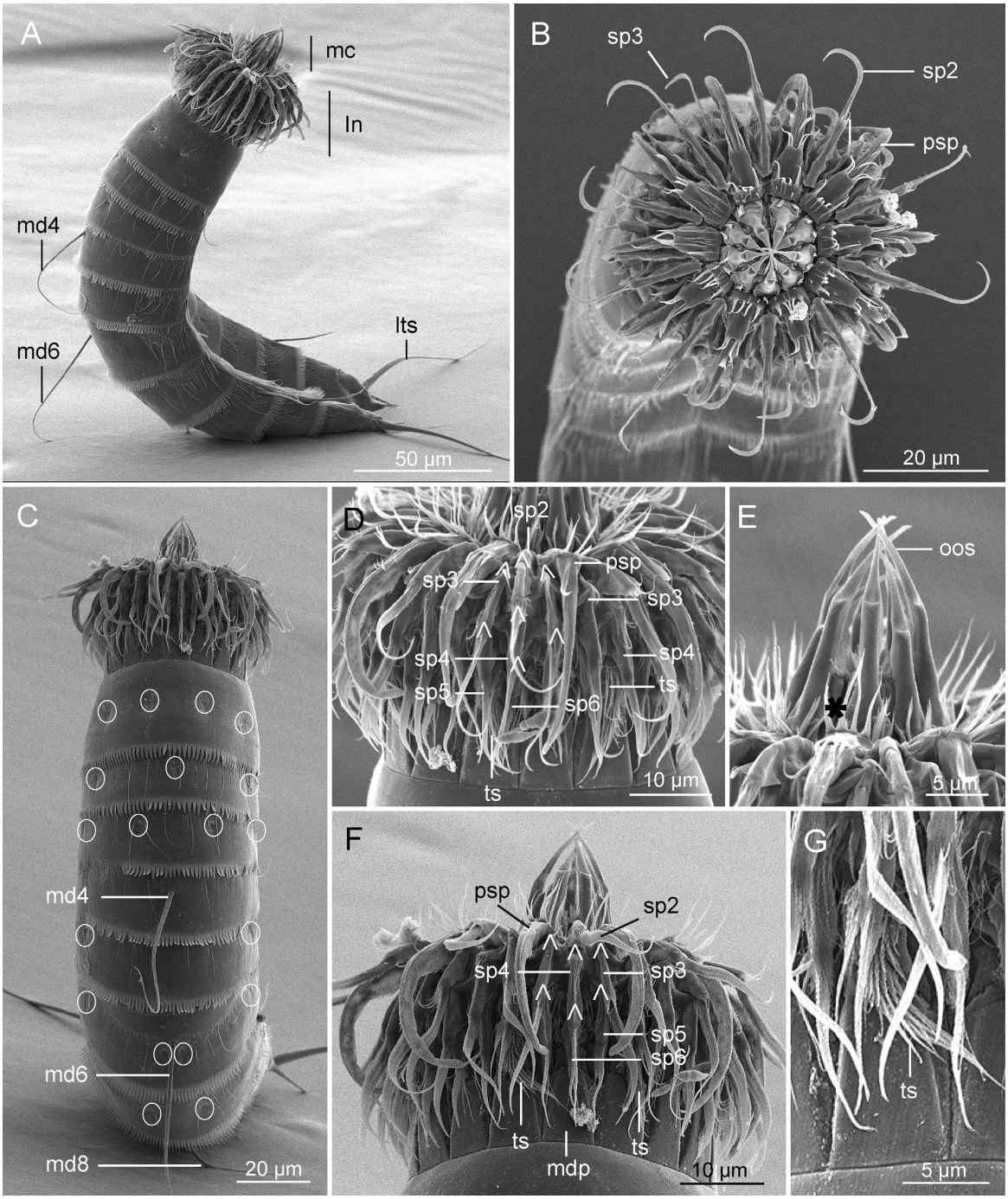


Fig. 9. Scanning electron micrographs showing introvert and trunk morphology in *Meristoderes boylei* sp. nov. (A) Male lateral overview. (B) Mouth cone and introvert, apical view. (C) Male, introvert, neck and segments 1–7, dorsal view. (D) Introvert showing sector 4. (E) Mouth cone, laterodorsal view. (F) Introvert showing sector 6. (G) Detail of a dorsal trichoscalid sector 7. *Abbreviations*: In, introvert; lts, lateral terminal spine; mc, mouth cone; md, middorsal spine; mdp, middorsal placid; oos, outer oral styles; psp, primary spinodcalids; sp 1–6, spinoscalids; numbers refer to the row; ts, trichoscalid. Lambda symbols (Λ) mark attachment points of spinoscalids. Asterisk marks middorsal position in the mouth cone. White circles indicate sensory spots. Digits after abbreviations refer to segment number.

(Figs. 6A and B, 8A and B and 9C). Sensory spots rounded and composed of numerous minute papillae with two pores. Dorsal sensory spots flanked by two long hairs with round perforation sites. Additional cuticular hairs absent, giving the segment a smooth appearance (Figs. 6A and B, 8A–C and 9A and C).

Segment 2 consists in a closed cuticular ring with partial tergosternal divisions (6 µm) arising from the pectinate fringe and reaching the middle of the segment close to the base of the ventrolateral tubules. They are clearly visible with SEM but less conspicuous when using LM (Figs. 8A and C and 10B).

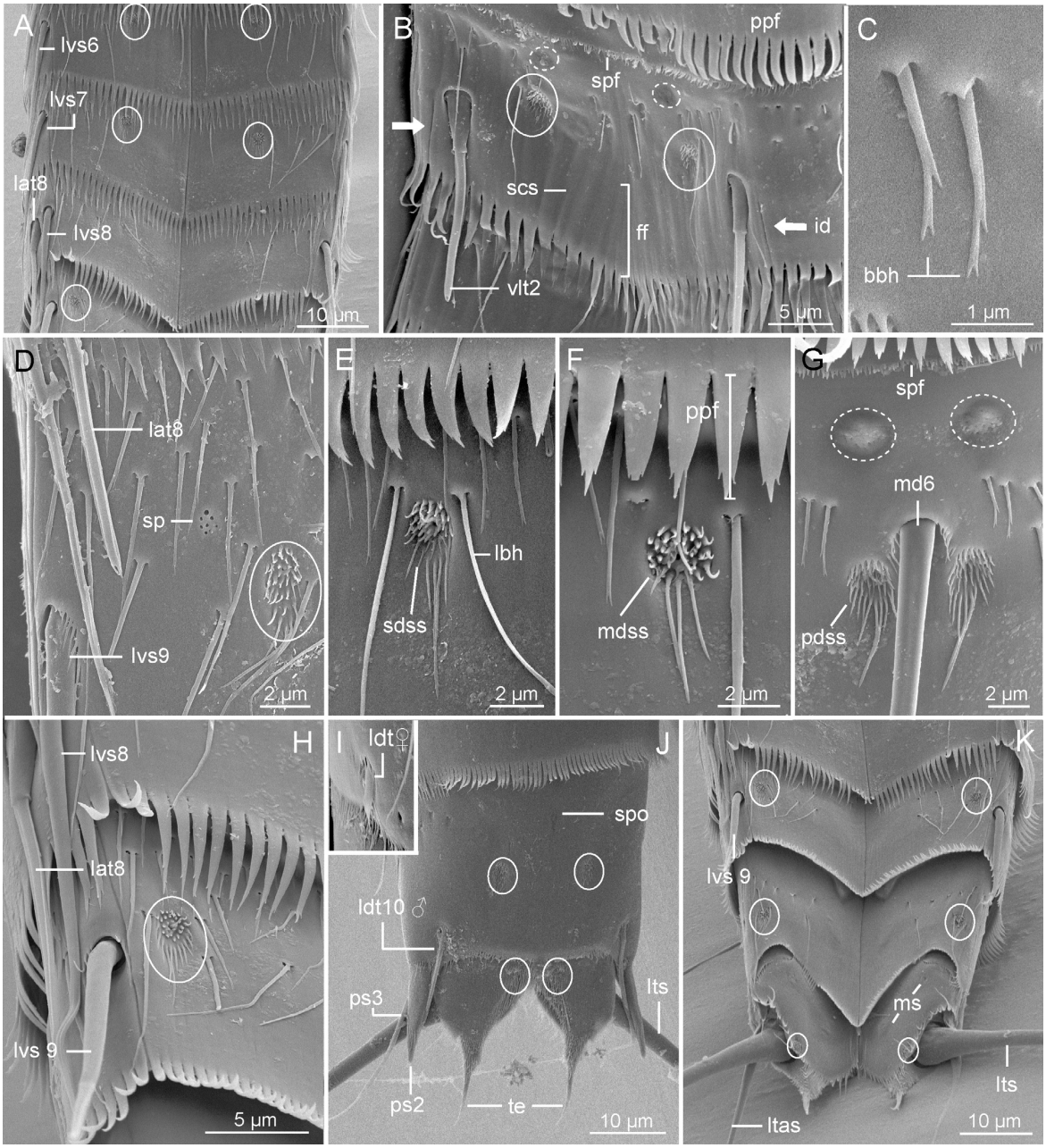


Fig. 10. Scanning electron micrographs showing trunk morphology and cuticular details in *Meristoderes boylei* sp. nov. (A) Segments 6–8, ventral view. (B) Segment 2, ventral view, note incomplete divisions (arrow). (C) Detail of bracteated branched hairs segment 6, dorsal view. (D) Detail of lateroventral to laterodorsal area on segment 9, lateral view. (E) Subdorsal sensory spot flanked by two long bracteated hairs, segment 3. (F) Middorsal sensory spot showing three enlarged papillae posteriorly, segment 2. (G) Detail of middorsal area on segment 6. (H) Ventrolateral areas of right sternal plates of segments 8 and 9. (I) Female laterodorsal tubule, segment 10. (J) Male segments 10 and 11, dorsal view. (K) Female segments 9–11, ventral view. *Abbreviations*: bbh, bracteated branched hairs; ff, free flap; id, incomplete division; lat, lateral accessory tubule; lbh, long bracteated hair; ldt, laterodorsal tubule; lts, lateral terminal spine; lvs, lateroventral spine; md, middorsal spine; mdss, middorsal sensory spot; ms, muscular scar; pdss, paradorsal sensory spot; ppf, primary pectinate fringe; ps1–3, penile spines; scs, subcuticular striation; sdss, subdorsal sensory spot; sp, sieve plate; spf, secondary pectinate fringe; spo, single pore opening; te, tergal extension; vlt, ventrolateral tubule. White circles indicate pore fields (dotted line) and sensory spots (solid line). Digits after abbreviations refer to segment number.

One pair of thick tubules is present in a ventrolateral position; each tubule consists of a short and smooth basal part, and a longer distal part with two small wing-like lateral projections (Figs. 8C and 10B). Paired sensory spots in laterodorsal and ventromedial positions plus an unpaired sensory spot in middorsal position (Figs. 6A, 8A, 9C and 10B). Ventromedial sensory spots rounded with a single long hair (perforation site) associated (Fig. 10B), laterodorsal and middorsal sensory spots flanked by two long bracteated hairs (Figs. 6B and 10E), middorsal sensory spot rounded with 3 longer papillae in the posteriormost area (Fig. 10F).

All cuticular hairs on this and all following segments are bracteated and branched being grouped in distinct clusters. The hair branches are more noticeable in short hairs rather than long ones (Fig. 10C). A cluster with 2 hairs is present paraventrally in each sternal plate of this segment; additionally a narrow band formed by two rows of short hairs surrounds the segment except for laterodorsal and ventromedial areas (Fig. 10B). Free flap as described on previous segment.

Segment 3 and the following 8 segments consist in one tergal and two sternal plates (Figs. 6A and B and 8A and B). Paired oval sensory spots with posteriorly elongated papillae flanked by two hairs, present in subdorsal and laterodorsal areas (Fig. 10E). Dorsal hair pattern as described on previous segment. A paraventral cluster of perforation sites arranged in 2 rows with the posteriormost row formed by 2 conspicuously long hairs present on each sternal plate in this and the following 3 segments (Figs. 6A and 10A).

Segment 4 with a long and flexible middorsal spine (Figs. 6B, 8B and 9A and C). No sensory spots present. Other characters, including paraventral hair clusters, similar to previous segment.

Segment 5 with a pair of long lateroventral tubules with the same appearance of those on segment 2 (Figs. 6A and 8A). Two pairs of oval sensory spots present in laterodorsal and ventromedial positions without associated hairs (Figs. 6A, 8A and 9C). Cuticular hairs arranged in 2 subdorsal, 2 laterodorsal and 2 ventrolateral clusters formed by 2–3 rows with the longest hairs located in the most posterior row of each cluster. Paraventral hair clusters as described in segment 4.

Segment 6 with a long and flexible middorsal spine and a pair of lateroventral spines (Figs. 6A and B, 8A and B, 9A and C and 10A and G). Paired sensory spots present in paradorsal, laterodorsal and ventromedial positions (Figs. 6A and B, 8A and B, 9C and 10G). All sensory spots are big and oval with no associated hairs (Fig. 10G). Paraventral hair clusters as described in segment 4. Other characters, including remaining cuticular hair patterns similar to those on the previous segment.

Segment 7 with a pair of long lateroventral spines (Figs. 6A, 8A and 10A). Paired oval sensory spots in subdorsal, laterodorsal and ventromedial positions; paradorsal pair with two associated hairs (Figs. 6A and B and 9C). Dorsal sensory spots showing very enlarged papillae posteriorly (Fig. 9C). Paraventral hair clusters composed by 2 rows of hairs, but without the conspicuously long hairs described in segments 3–6 (Fig. 10A). Cuticular hair arrangement otherwise similar to those on previous segments.

Segment 8 with a long and flexible middorsal spine flanked by a pair of oval paradorsal sensory spots (Figs. 6B, 8B and 9C). A pair of long lateroventral spines and an additional pair of long lateral accessory tubules with the same structure of those in segments 2 and 5 is present (Figs. 6A, 8A and D and 10A, D and H). No other sensory spots are present. Paraventral hair clusters as described in segment 7. Remaining cuticular hair arrangement similar to those on previous segments.

Segment 9 with shorter lateroventral spines (Figs. 6A and C, 8A and D and 10H and K). Paired oval sensory spots in paradorsal, subdorsal, laterodorsal and ventrolateral positions; the latter with two associated hairs (Figs. 6A and D and 10D, H and K). A single pore opening is present in middorsal position in between the paradorsal sensory spots (Fig. 10J). Paired small and rounded sieve plates composed of 8–10 pores are present in sublateral positions (Figs. 6B and 10D). Paraventral hair clusters as described in segment 7. Remaining cuticular hair arrangement similar to those on previous segments.

Segment 10, males with a pair of long laterodorsal tubes with the same structure that those described in segments 2, 5 and 8 emerging from fringed notches (Figs. 6B and 10J); females with shorter and soft tubules with fringed ends and without the typical structure showed in males (Figs. 6D and 10I). Paired oval sensory spots in subdorsal and ventrolateral positions (Figs. 6A–D and 10J and K). Ventrolateral sensory spots with long papillae protruding from the posterior part and two associated hairs similar to those on segment 9 (Fig. 10K). Posterior margin of the segment with a short pectinate fringe increasing in length toward the midventral region; fringe tips, thinner and flexible, are not trident-shaped (Fig. 10J and K). Free flap with a midventral extension covering the midventral anterior margin of segment 11. Perforation sites restricted to the lateral sides of the segment being very scarce paradorsally (Fig. 10J).

Segment 11 with one pair of very long lateral terminal spines exceeding the trunk length present in both sexes, females with an additional pair of lateral accessory spines (Figs. 6A–D, 8A, 9A and 10J and K). Males with three pairs of penile spines; p1 and p3 longer than p2, which is stout, shorter, many times thicker and with a triangular shape (Figs. 6A and B, 8E and 10J). Paired rounded sensory spots present in paradorsal, subdorsal and ventrolateral positions (Figs. 6A and B and 10J and K). Paradorsal sensory spots are larger situated adjacent to the middorsal fringed area of the tergal plate (Fig. 10J). Tergal extensions long, pointed and dorsally hairy with a distinct notch (Figs. 6A–C, 8E and 10J and K). Cuticular hair-like extensions and fringes covering the margins of the tergal plate (Fig. 10J). The posterior margin of the sternal plate is rounded with a long and flexible pectinate fringe (Fig. 10K). Scars from muscle attachments are present in ventromedial and ventrolateral positions (Fig. 10K).

3.2.6. Remarks

The combination of a first segment ring-shaped and a second segment showing incomplete tergosternal divisions clearly places the new species inside *Meristoderes*. Within *Meristoderes*, *M. boylei* sp. nov. shows the greatest resemblance with *M. macracanthus*. Both species share the same spine and tubule formula and therefore they could be confused. However, *M. boylei* sp. nov. can be discriminated by the presence of long and pointed tergal extensions; these are short and triangular in *M. macracanthus*. Both species also differ in body proportions, with the TL of *M. boylei* sp. nov. being around 20% longer than *M. macracanthus*. Also the remarkably long spines described in *M. macracanthus* accounting for the name of the species (from Greek *makro*, long +*akantha*, spine) are not that long in *M. boylei* sp. nov. Middorsal spines are around 10 μm shorter, whereas the lateroventral spines are the most distinguishable being nearly half of the length in *M. boylei* sp. nov. Lateroventral spines on segments 6, 7, 8 and 9 in *M. boylei* sp. nov. measure 27, 28, 33 and 30 μm respectively, compared with 44, 49, 53, 36 μm respectively in *M. macracanthus*. By contrast, lateral terminal spines are more than 100 μm longer in *M. boylei* sp. nov. than in *M. macracanthus*. Other than the spine length, both species differ at some details such as the pectinate fringe of segment 1, being almost smooth with a midventral serration in *M. macracanthus* but well-developed all around the segment and showing long fringe tips in *M. boylei* sp. nov. The sensory spot pattern shows many differences between both species as well (Table 4; compare with Table 3 in Herranz et al., 2012).

M. boylei sp. nov. is easily distinguished from all remaining species of *Meristoderes*: *M. galatheae* differs by the presence of a single, short middorsal spine on segment 4 opposed to spines on segments 4, 6 and 8. *M. elleae* differs by the absence of lateral accessory tubules on segment 8 and the presence of glandular cell outlets type 2 on segments 2, 6 and 8. *M. herranzae*, *M. imugi*, *M. glaber* and an undescribed species (see Sørensen et al., 2013) differ by the presence of additional tubules in the dorsal series of segment 2 and

additional or differently located tubules on segment 8. *M. glaber* and *M. imugi* also differ by the lateroventral spine location on segments 7–9 in the first and 8–9 in the second.

Below follows an updated dichotomous key for the identification of all the species belonging to the genus *Meristoderes* modified from Sørensen et al. (2013) and including the new species *M. boylei* sp. nov.

3.2.6.1. Dichotomous identification key to species of Meristoderes.

1.	Middorsal spines present on segments 4, 6 and 8	2
	Short middorsal spine present on segment 4 only	*M. galatheae*
2.	No tubules present in dorsal series of segment 2	3
	Tubules present in dorsal series (subdorsal or laterodorsal) of segment 2	4
3.	Tubules in lateral accessory positions on segment 8	7
	Glandular cell outlet type 2 present in lateral accessory position on segment 8, laterodorsal and sublateral positions on segment 2, and sublateral position on segment 6	*M. elleae*
4.	Lateroventral spines present on segment 6; tubules present in dorsal or lateral series on segment 85	
	Lateroventral spines absent on segment 6	6
5.	Segment 8 with two pairs of tubules, subdorsal and lateral accessory	*Meristoderes* sp.
	Segment 8 with one pair of lateral accessory tubules	*M. herranzae*
6.	Lateroventral spines on segments 7, 8 and 9	*M. glaber*
	Lateroventral spines only on segments 8 and 9; sublateral tubules on segment 8	*M. imugi*
7.	Tergal extensions long and pointed; very long lateral terminal spines, 112% of body length	*M. boylei* sp. nov.
	Tergal extensions short and triangular, lateral terminal spines 88% of body length	*M. macracanthus*

Within *Meristoderes* complete information on the introvert structure is only available for 2 out of 7 species; the introvert of *M. boylei* sp. nov. has been completely mapped and matches the introvert of *M. macracanthus* in number and arrangement of scalids. This is not surprising, considering the general resemblance between the two species. However, another recently discovered echinoderid species (Herranz et al., in press) shows exactly the same introvert composition. This can be indicative of either a very conservative structure and arrangement of introverts or that introvert arrangements are highly homoplastic.

Furthermore, as occurring in *F. sorenseni* sp. nov. and also in re-examined specimens of *M. macracanthus* and other *Echinoderes* species (Herranz, pers. obs.) a slight difference in length between basal parts in alternating outer oral styles was detected. In the future, it would be desirable to examine this feature in more species and genera, in order to evaluate its relevance, and eventually test if it is a general trait for all species of Echinoderidae.

Outside *Meristoderes*, *M. boylei* sp. nov. shares the spine pattern with *Echinoderes abbreviatus* Higgins, 1983; *Echinoderes higginsi* Huys and Coomans, 1989; *Echinoderes kristenseni* Higgins, 1985; *Echinoderes riedli* Higgins, 1978 and *Echinoderes wallaceae* Higgins, 1983 (see Higgins, 1978, 1983, 1985; Huys and Coomans, 1989). However, all these species can be easily discriminated from *M. boylei* sp. nov., not only by their composition of segment 2 (being a closed ring) but also by the trunk and spine dimensions, sensory spot pattern and appearance, and presence of glandular cell openings type 2.

4. Discussion

4.1. Geographic distribution

None of the genera treated in the present contribution have previously been reported from the Atlantic Ocean. Species of *Fissuroderes* were originally described from deep waters close to New Zealand and the continental shelf off the Pacific coast in Costa Rica (Neuhaus and Blasche, 2006). In turn, the type species of *Meristoderes* has been reported from the Mediterranean Sea (off the coast of Blanes, Spain; Naples and Sardinia, Italy) (Herranz et al., 2012) while the remaining six species were described from Pacific waters (Korean coasts and the Solomon Islands) (Herranz et al., 2012; Sørensen et al., 2013). The discovery of both genera in the Atlantic Ocean enlarges their distributional range, which points to be worldwide. In case of *Meristoderes* this is not surprising, knowing the presence of the genus in Mediterranean waters. The finding of *Meristoderes* in East Atlantic waters would also be expected having in mind the presence of American and European species (see below). For *Fissuroderes*, all known species have only been reported once and seem to be quite local, only appearing in single or closely located positions, except for *F. higginsi* that appeared in more distant locations and very different depths (Neuhaus and Blasche, 2006). Within *Meristoderes*, the special conditions of the formation and geological evolution of the Mediterranean Sea together with the close resemblance between the Mediterranean *M. macracanthus* and the Atlantic *M. boylei* sp. nov. suggest that the species are closely related. The Messinian crisis, around 6 million years ago, was caused by reduced water inflow from the Atlantic Ocean to the Mediterranean Sea. This resulted in a widespread salt precipitation and a decrease in Mediterranean Sea level of about 1.5 kilometers due to evaporation (García-Castellanos and Villaseñor, 2011). Within one millennium the Mediterranean basin was nearly completely desiccated, leaving only a few hypersaline areas. Messinian salinity crisis had an almost sterilizing effect in the marine invertebrate life in the Mediterranean Sea, perhaps with very few exceptions (Loricifera, see Danovaro et al., 2010). The Mediterranean refill and therefore the repopulation of most marine animals came from the Atlantic through the Gibraltar Strait in a surprisingly short period of 2 years (García-Castellanos et al., 2009). This theory can explain the great resemblance between *M. macracanthus* from the Mediterranean Sea and *M. boylei* sp. nov. from the West Atlantic Ocean, that at the Messinian crisis was closer to the Mediterranean than today. Both species could have diverged from the same Atlantic ancestor and evolved independently in each area following allopatric speciation. Currently both species still share many characters that may be explained by a low rate of evolutionary change, as pointed out by Neuhaus and Sørensen (2013) for *Campyloderes*.

The knowledge of the global distribution of Kinorhyncha is still very limited, and could be reduced to the random distribution of sampled localities. Except for the Iberian Peninsula, Korea, the NW and East coasts of USA (see Higgins, 1964, 1965, 1982, 1990; Lundbye et al., 2011; Sørensen, 2007; Sørensen et al., 2005, 2007, 2012a,b, 2013; Sánchez et al., 2012), large areas and/or long coastlines have rarely been subject to extensive and systematic sampling. Indeed we expect to find in the future more specimens and species of both *Fissuroderes* and *Meristoderes* in Atlantic and even in Mediterranean waters.

4.2. Considerations on the Family Echinoderidae

Currently the family Echinoderidae accommodates 5 genera: *Meristoderes*, *Polacanthoderes*, *Echinoderes*, *Cephalorhyncha* and *Fissuroderes*. Echinoderidae was monogeneric until the description of the genus *Cephalorhyncha* (Adrianov, 1999). Since then, three new genera have been assigned to the family: *Fissuroderes*, *Polacanthoderes* and *Meristoderes*. Until the description of *Polacanthoderes* in 2008 (Sørensen, 2008a), the presence of the second trunk segment composed of one tergal and two sternal plates was an autapomorphic feature of *Fissuroderes* within Echinoderidae. Indeed, the plate arrangement of the second trunk segment has been the key character to discriminate the different genera inside the family (see Fig. 11 in Herranz et al., 2012). Subsequently, the erection of the

monospecific genus *Polacanthoderes* somehow complicated the taxonomic situation inside Echinoderidae (see Discussion in Sørensen, 2008a). The actual relationships within the group, with the establishment of reliable autapomorphies are to be settled in the future through a deep phylogenetic analysis following the steps of Sørensen (2008b) and Yamasaki et al. (2013). The latter is the only molecular study dealing with internal relationships of the phylum; however, the lack of data from the three newest mentioned genera prevented any conclusion. Enlarged and updated datasets, with consideration of the missed genera and combining new morphological and molecular characters are the needed approach for further studies to come.

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Morphology

Chapter VI

Comparative myoanatomy of *Echinoderes* (Kinorhyncha): A comprehensive investigation by CLSM and 3D reconstruction

Anatomía muscular comparada en *Echinoderes*.
Una investigación detallada a través de la microscopía
confocal láser y la reconstrucción tridimensional

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Los kinorrincos constituyen un clado de invertebrados marinos de la meiofauna. Su modelo de organización consiste en un introverto retráctil con anillos de espinas cuticulares y un tronco sin apéndices con una clara segmentación de los sistemas nervioso, muscular y epidérmico. Como miembros basales de los Ecdisozoos, los kinorrincos enlazan con la evolución y radiación de la segmentación en uno de los tres grandes superclados de los animales bilaterales. En este trabajo se describe la mioanatomía de *Echinoderes,* el género de kinorrincos con mayor número de especies, construido sobre estudios históricos de ultraestructura y morfología en kinorrincos. Este trabajo constituye el primer estudio comparado en múltiples especies de un sistema orgánico completo mediante microscopía confocal y reconstrucción tridimensional en el filo Kinorrincos.

La mioanatomía de los individuos adultos de *Echinoderes* está compuesta por: Una cabeza con dos músculos circulares del cono bucal, nueve pares de músculos de los estiletes orales, diez retractores del introverto, un músculo circular del introverto y catorce músculos retractores del músculo circular; un cuello con un músculo circular; un tronco con pares de músculos dorsales y ventrales en los segmentos 1-10, músculos dorsoventrales en los segmentos 3-10, músculos diagonales en los segmentos 1-8, fibras longitudinales que se extienden del segmento 1 al 9, tres pares de músculos de las espinas terminales y un par de músculos de las espinas peneanas en los machos; el tubo digestivo presenta una faringe con diez anillos musculares que alternan en disposición radial y circular, rodeados por una compleja vaina de protractores y retractores, una red ortogonal de fibras longitudinales y circulares rodeando al intestino, y músculos pares dilatadores del digestivo posterior.

La mioanatomía de los individuos adultos de *Echinoderes* está muy conservada entre las especies. Se observa variación intraespecífica en el número y disposición de las fibras del introverto y en la composición de los músculos faríngeos. La musculatura segmentaria del tronco facilita la articulación de las placas cuticulares a lo largo del eje anteroposterior. Las fibras musculares intersegmentarias contribuyen a los movimientos dorsoventrales y laterales del tronco. Los músculos protractores, retractores y circulares coordinan la eversión y retracción del introverto y el cono bucal, y la recolocación de la faringe durante la locomoción y la alimentación. Las fibras posteriores pares sugieren el movimiento independiente de las espinas terminales y la actividad reproductora de las espinas peneanas de los machos. Dentro de los Escalidóforos, la mioanatomía es más similar entre Kinorrincos y Loricíferos que entre cualquiera de estos y los Priapúlidos. La mioanatomía de los kinorrincos puede reflejar una antigua transición entre los planes corporales vermiforme y segmentado durante la radiación temprana de los Ecdisozoos.

Comparative myoanatomy of *Echinoderes* (Kinorhyncha): A comprehensive investigation by CLSM and 3D reconstruction

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Abstract

Introduction: Kinorhyncha is a clade of marine invertebrate meiofauna. Their body plan includes a retractable introvert bearing rings of cuticular spines, and a limbless trunk with distinct segmentation of nervous, muscular and epidermal organ systems. As basal members of Ecdysozoa, kinorhynchs provide a link to the evolution and radiation of segmentation within one of three major bilaterian superclades. Here, we describe the myoanatomy of *Echinoderes*, the most specious kinorhynch genus, and build upon historical studies of kinorhynch ultrastructure and gross morphology. This is the first multi-species comparison of a complete organ system by confocal microscopy and three-dimensional reconstruction within Kinorhyncha.

Results: Myoanatomy of adult *Echinoderes* is composed of the following: Head with two mouth cone circular muscles, nine pairs of oral style muscles, ten introvert retractors, one introvert circular muscle, and fourteen introvert circular muscle retractors; Neck with one circular muscle; Trunk showing distinct pairs of ventral and dorsal muscles within segments 1-10, dorsoventral muscles within segments 3-10, diagonal muscles within segments 1-8, longitudinal fibers spanning segments 1-9, three pairs of terminal spine muscles, and one pair of male penile spine muscles; Gut showing a pharynx with ten alternating rings of radial and circular muscle fibers enclosed in a complex sheath of protractors and retractors, an orthogonal grid of longitudinal and circular fibers surrounding the intestine, and paired hindgut dilators.

Conclusions: The myoanatomy of Adult *Echinoderes* is highly conserved between species. Interspecific variation is observed in the arrangement and number of introvert fibers and the composition of pharyngeal muscles. Segmented musculature of the trunk facilitates the articulation of cuticular plates along the anterior-posterior axis. Intersegmental muscle fibers assist with dorsoventral and lateral trunk movements. Protractors, retractors and circular muscles coordinate eversion and retraction of the introvert and mouth cone, and relocation of the pharynx during locomotion and feeding behaviors. Pairs of posterior fibers suggest independent movements of terminal spines, and reproductive activity of male penile spines. Within Scalidophora, myoanatomy is more similar between Kinorhyncha and Loricifera, than either group is to Priapulida. Kinorhynch myoanatomy may reflect an ancient transition from vermiform to segmented body plans during the early radiation of Ecdysozoa.

Keywords: Ecdysozoa, Scalidophora, segmentation, phalloidin, organ system, circular muscle, introvert, pharynx.

Introduction

Kinorhyncha is a clade of invertebrate meiofauna that inhabit marine sediments from the intertidal zone to abyssal depths in all major ocean basins [1]. They have a distinctly segmented body plan, with a retractable introvert and a diverse array of cuticular structures along its length. Very little is known about their embryonic development [2], there are no larval life history stages, and only a few studies have examined growth and morphogenesis in pre-adult stages [3, 4, 5]. The origin of Kinorhyncha and the relationships within the clade are unresolved. Within Metazoa, kinorhynchs are members of the 'moulting animals' known as Ecdysozoa [6], one of two protostome superclades [7-10]. And within Ecdysozoa, the kinorhynchs share a branch with priapulids, and most likely loriciferans, as the most basal ecdysozoan clade, the Scalidophora [6, 11-14]. Near that base, there are molecular and morphological similarities between Kinorhyncha and other cycloneuralians (i.e., priapulids, loriciferans, nematodes, nematomorphs), some of which are thought to be phylogenetically insightful. However, the Cycloneuralia also include a diversity of unsegmented vermiform taxa (e.g., nematodes), and monophyly has not been established for this group [9, 10, 15, 16]. Because of this, the assignment of particular synapomorphic characters within major organ systems, including digestive, nervous, and muscular tissues, must also remain tentative [14, 17, 18]. Yet, regardless of when and where particular branching patterns and prospective characters finally stabilize, kinorhynchs are the most basal ecdysozoan animals with segments [5, 19, 20], and therefore represent a key linkage in the evolution of segmentation between all extant ecdysozoans and the ancestral body plans from which they arose.

The trunk region of adult kinorhynchs consists of eleven well-defined segments, reflecting an anterior-posterior pattern of repeated elements in the epidermis, nervous system and musculature. Most notable are eleven sets of articulating exoskeletal plates, a ganglionated ventral nerve cord with perpendicular branching of neurites to the peripheral nervous system, and paired sets of functionally specific muscle bands along the trunk [1, 21]. Although developmental studies are significantly absent in Kinorhyncha, it is reasonable to assume that such patterns include both ectodermal (exoskeleton, nervous system) and mesodermal (muscle) origins. Unlike most bilaterians, especially soft-bodied invertebrates, kinorhynchs do not develop the typical set of outer circular and inner longitudinal muscle groups below the epidermis, which may reflect the production of hard cuticle in their segmented exoskeleton [17]. The continuous circular and longitudinal muscle layers of the body wall have apparently been replaced by isolated muscles attached to the exoskeleton through an intermediate epidermal cell [1, 17, 22]. Moreover, kinorhynchs do not have external appendages along the body, unlike all of the segmented taxa of Panarthropoda [23]. Perhaps the absence of segment-specific appendage development in kinorhynchs is evidence of an ancient transition from vermiform to segmented body plans that are visible in most ecdysozoans, but missing among all other cycloneuralians. Thus, kinorhynchs are not only pivotal for understanding the evolution of segmentation, but also the radiation of unsegmented worms near the base of Ecdysozoa. And, moving further up into the tree, important similarities in muscle organization between kinorhynchs and arthropods suggest there is still a lot work to do. Studies of gene expression during segmentation, at analogous sites of appendage development, and within many other organ systems along the kinorhynch body are certainly needed, but do not exist. Until then, it is not possible to infer homology of muscles, nerves or even segments between kinorhynchs and other ecdysozoans with any certainty, at least not beyond predictions based solely on cladistics [5, 10, 23-25].

Apart from segmentation, kinorhynchs share several notable characteristics with their scalidophoran relatives, including a retractable introvert with rings of chitinous scalids, and the complex myoanatomy that operates it. Within the myofibrils of kinorhynch muscle cells, the sarcomeres are cross-striated. The exception is an orthogonal grid-like pattern of minute fibers encircling the kinorhynch intestine [5, 22, 26, 27]. Sarcomeres of this type, although not exclusive to the clade, are thought to facilitate rapid contractions of muscle fibers. As scalidophorans live among the sand grains of marine sediments, and do not have appendages (excluding the toes of the loriciferan Higgins larvae) or locomotory cilia [17, 18, 28], they must rely on introvert musculature for locomotion and feeding [29-32]. The introvert also thought to have important sensory roles in that environment [1, 11, 30, 33-35].

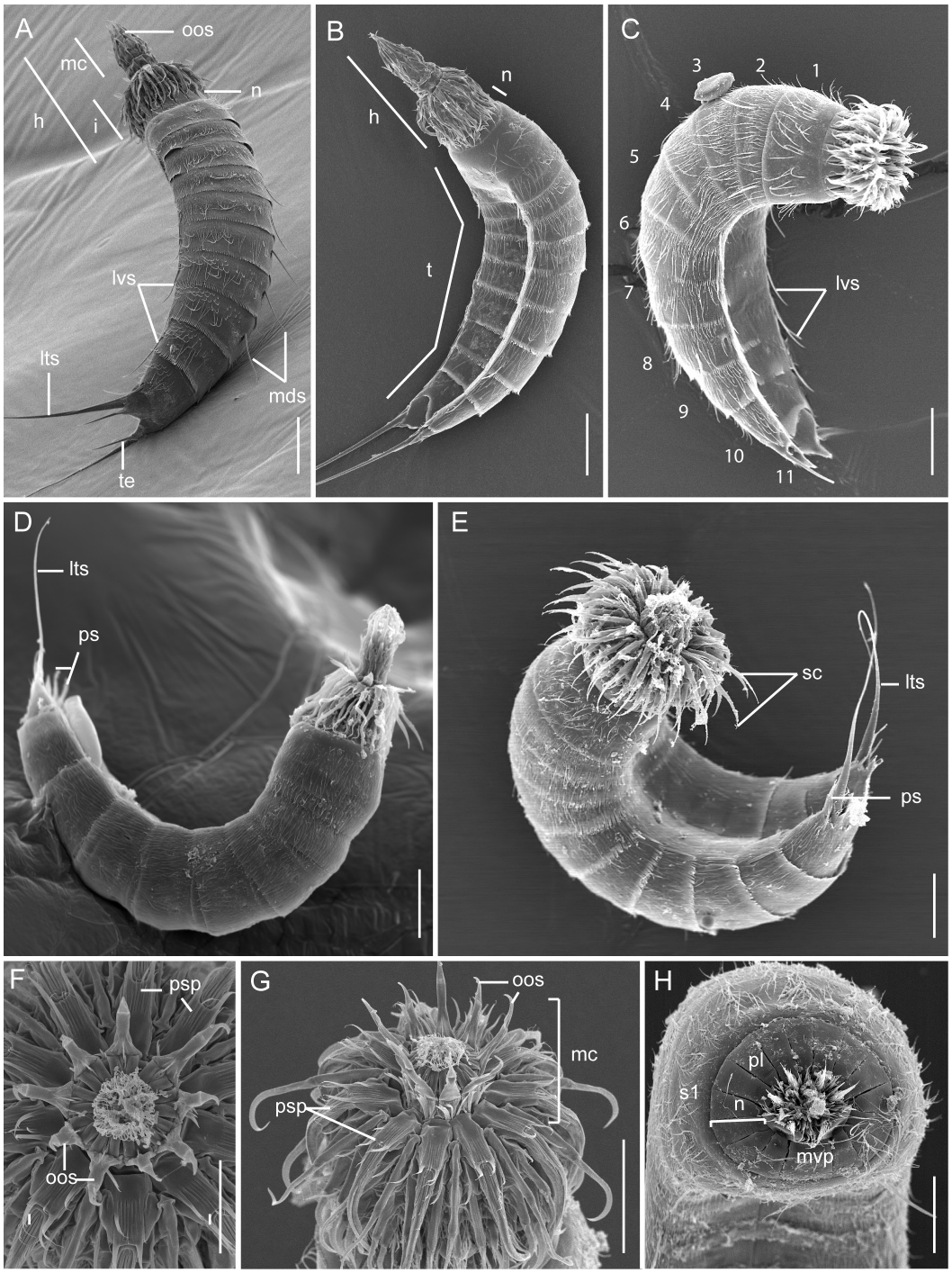

Figure 1 The external morphology of five species of *Echinoderes* (Kinorhyncha).
(A-H) Scanning Electron Microscopy (SEM). **(A)** *Echinoderes spinifurca,* lateral view, with ventral to the left, anterior to the top. Introvert and mouth cone are everted. **(B)** *Echinoderes horni,* lateroventral view, anterior to the top. Introvert and mouth cone are everted. **(C)** *Echinoderes hispanicus,* lateroventral view, anterior to the right. Introvert is everted, and the mouth cone is retracted. **(D)** *Echinoderes* sp., lateral view, with anterior and posterior toward the top, dorsal to the bottom. Introvert and mouth cone are everted. **(E)** *Echinoderes dujardinii,* lateroventral view, anterior and posterior toward the top. Introvert is everted and part of mouth cone is visible. **(F)** *Echinoderes spinifurca,* apical view of the mouth cone. **(G)** *Echinoderes spinifurca,* lateral view of the introvert and mouth cone, anterior to the top. **(H)** *Echinoderes dujardinii,* apical view. Introvert and mouth cone are retracted, and placids of the neck are closed. Abbreviations: h, head; i, introvert; lts, lateral terminal spine; lvs, lateroventral spine; mc, mouth cone; mds, middorsal spine; mvp, midventral placid; n, neck; oos outer oral style; pl, placid; ps, penile spine; psp, primary spinoscalid; s, segment; sc, scalids; t, trunk; te, tergal extension. Digits refer to segment number. Scale bar, 30 µm.

Furthermore, in each taxon, there are well-developed retractors, protractors, circular muscles, pharynges, and an assortment of external chitinous spines, teeth or scalids [1, 18, 30, 33]. Variation in character states for these and other features are not subtle, and appear to correspond with size, shape, behavior and the presence or absence of a hard exterior cuticle [1, 17, 36, 37]. Overall, similarities and differences in the function and presumed evolution of characters among kinorhynchs, within Scalidophora, and across Cycloneuralia, are not well understood. Yet, almost all of the internal anatomy, and many external structures, are thought to be directly associated with contractile cells and their fibers [1, 13, 17, 33, 38-40]. Thus, thorough descriptions of myoanatomy are essential.

Zelinka [41] and Remane [42] introduced the earliest information on kinorhynch musculature by light microscopy [41-42], which was followed ~ 60 years later by descriptions of ultrastructure using transmission electron microscopy [1, 13, 30, 33]. More recently, kinorhynch musculature was examined by a combination of cytochemical staining and confocal laser scanning microscopy (CLSM) in the cyclorhagid, *Antygomonas* sp. [26], and homalorhagid, *Pycnophyes kielensis* [5, 27]. Although each confocal study was limited in scope, and to a single species, musculature was selectively labeled and examined as an organ system, setting up the first comparisons between kinorhynchs and other ecdysozoans, and metazoans in general. In this manuscript, we present a comprehensive description of the muscular organ system in *Echinoderes*, the largest kinorhynch genus with nearly 80 species and a global distribution. We have selected five species, from both sides of the Atlantic Ocean, that exemplify morphological variability within the genus based upon a range of taxonomic characters: *Echinoderes horni* Higgins, 1983 [43]; *Echinoderes spinifurca* Sørensen et al., 2005 [44]; *Echinoderes dujardinii* Claparède, 1863 [45]; *Echinoderes hispanicus* Pardos Higgins and Benito, [46] and *Echinoderes* sp. (see Table 1). Thus far, the myoanatomy of *Echinoderes* has not been investigated with confocal laser scanning technology. Therefore, we have utilized a comparative multi-species approach, and have rendered the first complete three-dimensional reconstructions of kinorhynch musculature with 3D imaging software. Particular emphasis has been placed on the arrangement and function of muscles within the introvert, pharynx and associated structures at the anterior end of the kinorhynch body plan. Results of our investigation supplement previous studies with new observations and interpretations, and enable a broader discussion of comparative myoanatomy within Kinorhyncha, and between kinorhynchs and closely related groups, the Loricifera, Priapulida and Nematomorpha.

Results

External morphology of *Echinoderes*

As observed in all known kinorhynch genera, species of *Echinoderes* exhibit a bilaterally symmetric body plan that is divided into three regions along the anterior-posterior axis: head, neck and trunk (Figure 1A-E). The head region is unsegmented, and is composed of an eversible introvert with a retractable mouth cone. The introvert bears seven concentric rings of cuticular appendages, the scalids, which encircle the mouth cone; the scalids from the first ring are named primary spinoscalids (psp) (Figures 1A-B, G; 2A-F). The mouth cone includes nine articulated outer oral styles (Figure 1F, G), and three rings of inner oral styles that surround a centralized mouth within the terminal end of the cone. The neck region is also unsegmented, and is composed of sixteen cuticular plates, the placids. All of the placids are interconnected by soft cuticle, and they form a closing system for the anterior end of the trunk when the introvert is retracted (Figure 1H). The trunk is divided into eleven articulating segments (Figure 1A-E). The first and second segments are ring-like, however, segments 3-11 are each composed of one tergal (dorsal), and two sternal (ventral) plates. Particular trunk segments may have relatively long, cuticular spines extending from the surface of their plates. Spines typically extend in a posterior direction from a middorsal and/or lateroventral surface, or from the terminal end of segment 11 (Figure 1 A-E). Males have three pairs of penile spines (Figure 1D-E), and females possess a single pair of lateral terminal accessory spines. The primary differences in external morphology among species of *Echinoderes* include the size, number, and arrangement of cuticular spines, as well as notable variation in the presence and relative positions of other cuticular structures such as sensory spots, tubes, hairs and glands. Moreover, the length and proportion of trunk regions are highly variable

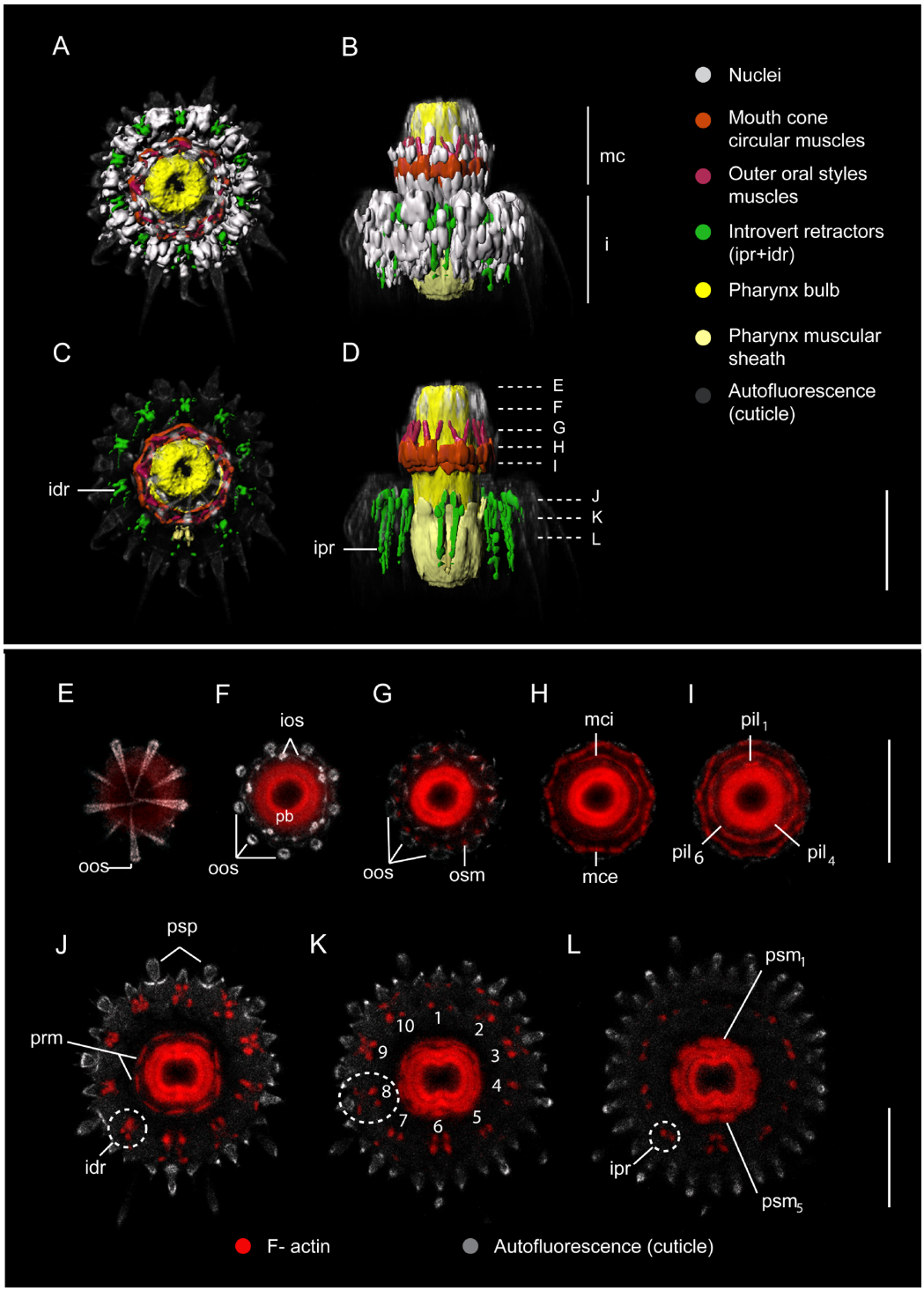

Figure 2 Myoanatomy of the mouth cone and introvert of *Echinoderes spinifurca*. (A-D) 3D reconstructions of confocal z-stacks in the head region. **(E-L)** confocal z-stack projections of phalloidin staining in the head region. **(A, C)** apical view, ventral side is down. **(B, D)** ventral view, anterior to the top. Cell nuclei are shown in A and B, but not shown in C and D for clarity. **(E-L)** Phalloidin staining in the same specimen as in A-D. Series of optical cross-sections by depth through the head as indicated by dashed lines in D; ventral side is down in all images. E-I, mouth cone region; J-L, introvert region. Numbers in K refer to the ten introvert retractors. Note: introvert scalids do not contain musculature. Abbreviations: idr, introvert distal retractors; ipr, introvert proximal retractors; ios, internal oral style; mce, mouth cone external circular muscle; mci, mouth cone internal circular muscle; oos, outer oral style; osm, outer oral style muscle; pb, pharynx bulb; pil, pharynx inner longitudinal muscle; prm, pharynx retractor muscle; psm, pharynx sheath muscle; psp, primary spinoscalids. Scale bars, 30 µm.

among species.

Myoanatomy of Echinoderes

A comparison among the five species of *Echinoderes* in this study reveals a similar pattern of myoanatomy, regardless of detectable differences in external morphology (Table 1, Figures 1A-E). For clarity, we divide our descriptions of musculature into head, neck, trunk and gut regions of the kinorhynch body plan. When observed, species-specific differences in myoanatomy are noted for a particular region**.**

Musculature of the head: mouth cone

Myoanatomy of the mouth cone includes longitudinal and circular muscle groups. There are eighteen short longitudinal muscles, arranged in nine pairs, situated at the bases of the outer oral styles (oos) (Figure 2G). These are the outer oral styles muscles (osm). One pair (osm) is located at the base of each outer oral style (Figures 2B, D, G; 3A-C), and the two longitudinal muscles of a single pair converge at their anterior end to form an inverted V-shape (Figure 2B, D). One V-shaped pair of muscles (osm) is associated with one outer oral style (oos) (Additional file 1, Figure 3A).

There are two concentric rings of mouth cone circular muscles, one external and one internal. Each muscular ring is composed of three fibers, which are located within the basal part of the mouth cone (Figures 2A-D, H, I; 3C; 4C, F, I). The external circular muscle (mce) is positioned along the bases of the outer oral styles, and oral styles muscles (Figures 2D, H; 3C). The internal circular muscle (mci) is situated basally to the inner oral styles, and likely associated with the pharynx (Additional file 1, Figures 2H-I, 3C). Both external and internal circular muscle rings appear to be attached to the soft cuticle in between the bases of their respective oral styles (Figure 2H-I). This is more noticeable in the external circular muscle, which shows a polygonal shape in cross section (Figure 2H). The three rows of inner oral styles are not associated with a myoanatomical feature other than the mouth cone internal circular muscle described above (Figure 2F). The external and internal circular muscles move independently from each other, and may be observed at different levels along the anterior-posterior axis of the mouth cone (Figures 2D; 4C, F, I; 5A-F).

Musculature of the head*: *introvert

The myoanatomy of the introvert includes a set of longitudinal introvert retractors, an introvert circular muscle, and a set of longitudinal introvert circular muscle retractors.

The introvert retractors are composed of one or two sets of longitudinal muscles.

There are ten groups of 2-3 short, thin distal retractor muscles (idr) that probably insert into the anterior part of the introvert (Figures 2C, J, K; 4B-I; 5D-F). These (idr) muscles alternate positions with the primary spinoscalids (psp) and bend toward the bases of the spinoscalids (Additional file 1, Figure 2C-D, J). The spinoscalids themselves do not possess musculature (Additional file 1, Figure 2C-D, J, K).

The distal retractor muscles (idr) are connected with ten pairs of longitudinal proximal retractor muscles (ipr), which extend in an anterior-posterior direction between the pharynx and the trunk musculature (Additional file 1, Figures 2D, L; 4B, E, H; 5 D-F). Each muscle in a pair of proximal retractor muscles (ipr) is composed of 2-3 fibers. The posterior ends of the proximal introvert retractors attach dorsolaterally or ventrolaterally, most likely at the segmental pachycycli (cuticular thickening situated at the anterior margin of a segment) from trunk segment 3 to segment 5 (Figures 4B, E, D, G; 5D-F).

In *E. dujardinii*, there is no distinction between proximal and distal retractor muscles. Instead, there are ten pairs of long continuous introvert retractors (ir) composed of four fibers each. These introvert retrac-

Table 1 Comparative difference in size, shape, and spines in five species of *Echinoderes*

	Trunk length (μm)	Outline	Middorsal spines	Tergal extensions
E. dujardinii	350	Barrel shaped	5 short	Short
E. hispanicus	300	Elongated	3 long	Short
E. horni	270	Elongated	None	Short
E. spinifurca	270	Elongated	5 long	Long
Echinoderes sp.	270	Bulbous anterior end	1 short	Short

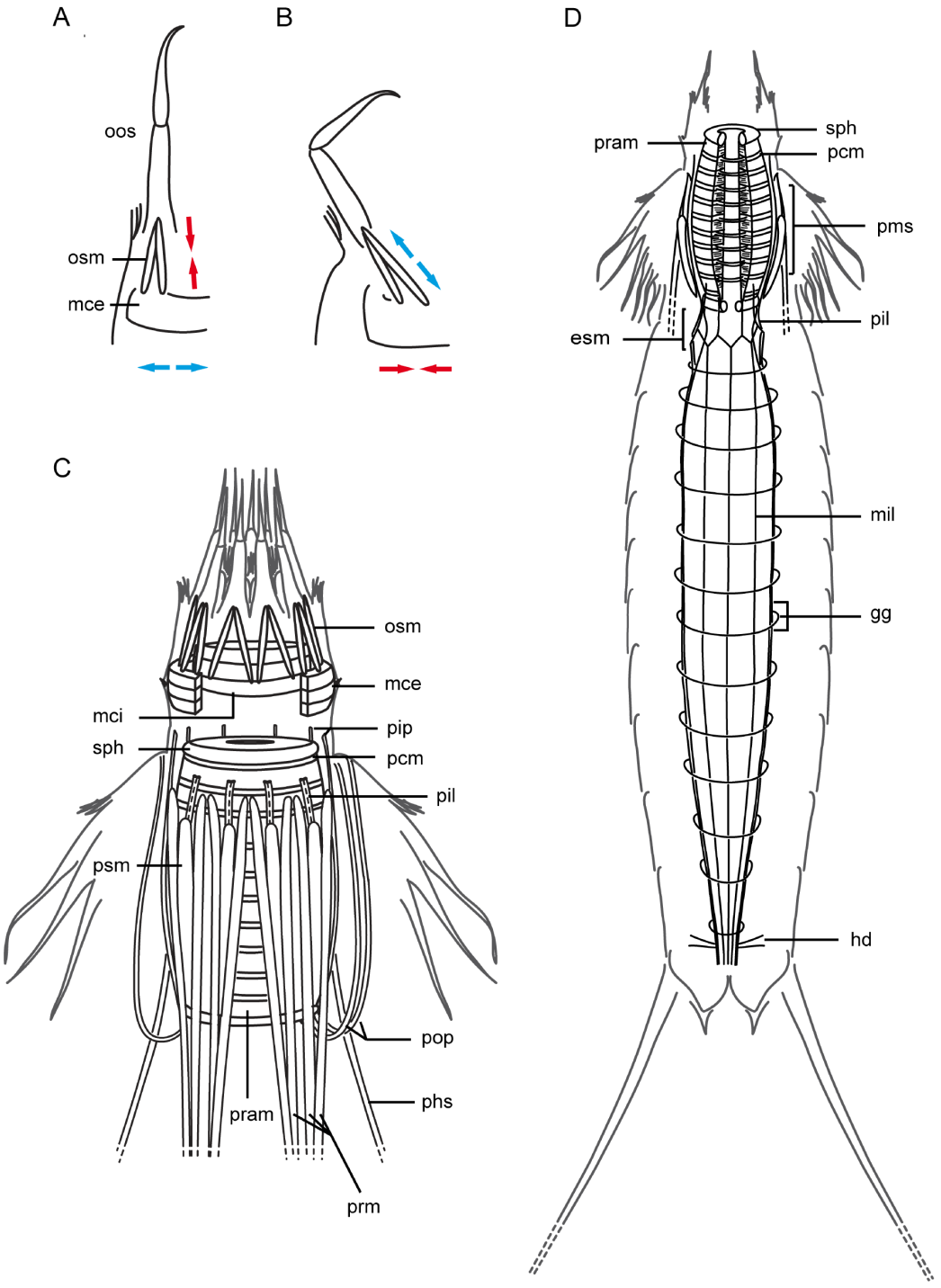

Figure 3 Schematics of musculature within subregions of the gut in *Echinoderes*.
(A-B) predicted antagonistic functions between muscles associated with the outer oral styles and the mouth cone external circular muscle. **(C)** myoanatomy of the mouth cone and pharynx in lateral view, with ventral side to the right. **(D)** overview of myoanatomy in the pharynx and intestine, in ventral view, with anterior to the top. Abbreviations: esm, esophagus muscles; gg, gut grid; hd, hindgut dilators; mce, mouth cone external circular muscle; mci, mouth cone internal circular muscle; mil, midgut inner longitudinal muscle; oos, outer oral style; osm, outer oral styles muscles; pcm, pharynx circular muscle fibers; phs, pharynx suspensor; pil, pharynx inner longitudinal muscle; pip, pharynx internal protractor; pms, pharynx muscular sheath; pop, pharynx outer protractor; pram, pharynx radial muscle fibers; prm, pharynx retractor muscle; psm, pharynx sheath muscles; sph, sphincter.

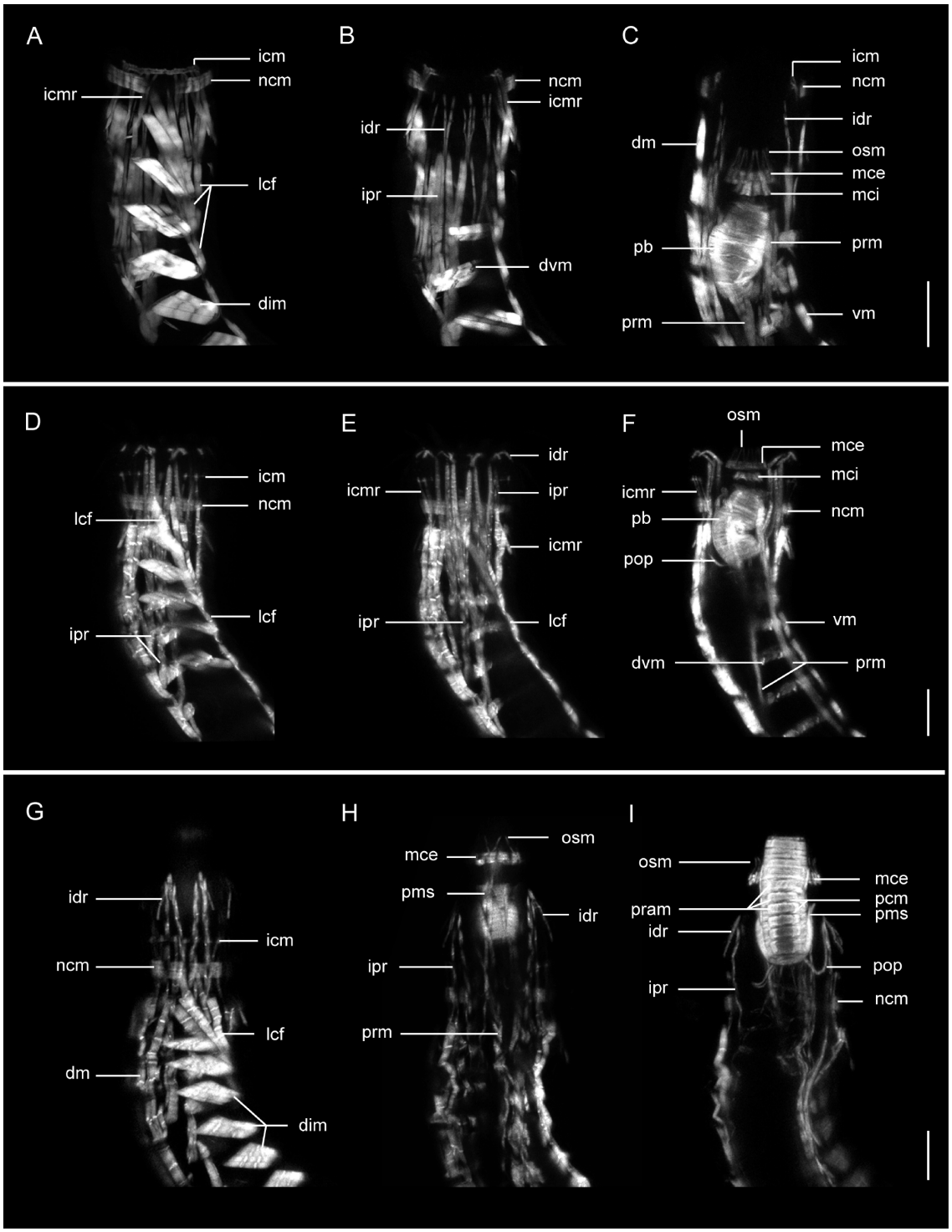

Figure 4 Musculature of the anterior regions in three species of Echinoderes. Confocal z-stack projections of phalloidin labeling. Three sets of images (A-C; D-F; G-I) each show a species-specific series of consecutively deeper z-stacks toward the pharynx. Anterior is to the top in all images. **(A-C)** *Echinoderes horni,* head retracted. **(D-F)** *Echinoderes hispanicus,* head partially extended. **(G-I)** *Echinoderes spinifurca*, head fully extended. Abbreviations: dm, dorsal muscle; dim, diagonal muscle; dvm, dorsoventral muscle; icm, introvert circular muscle; icmr, introvert circular muscle retractor; idr, introvert distal retractor; ipr, introvert proximal retractor; lcf, longitudinal continuous fiber; mce, mouth cone external circular muscle; mci, mouth cone internal circular muscle; ncm, neck circular muscle; osm, outer oral style muscle; pb, pharynx bulb; pcm, pharynx circular muscle fibers; pip, pharynx internal protractor; pop, pharynx outer protractor; pms, pharynx muscular sheath; pop, pharynx outer protractor; pram, pharynx radial muscle fibers; prm, pharynx retractor muscle; vm, ventral muscle. Scale bar, 20 µm.

tors attach posteriorly in the same trunk segments as in the other *Echinoderes* species, and bend anteriorly in a swan-neck-shaped configuration toward the bases of the spinoscalids (Figure 5A-C). Only the two central fibers, out of four fibers, bend toward the spinoscalids. The remaining two short fibers are positioned adjacent to the longer, curved fibers.

The introvert circular muscle (icm) is composed of 3-5 thin fibers, and its position changes depending on the position of the introvert (Figure 6). When the introvert is everted, the introvert circular muscle is relaxed and situated at the level of the last row of spinoscalids (Figures 4D-I, 6A). In this position, the diameter of this circular muscle is larger than the diameter of the neck (Figures 4D-F, 6A). However, when the introvert is fully retracted, the introvert circular muscle is constricted to a smaller diameter (Fig 4A-C, 6C) and situated below the level of the neck, within the anterior region of the first trunk segment (Figure 6C). There are approximately 14-16 short longitudinal muscles that are inserted into the introvert circular muscle (Figure 6); their anterior ends are bifurcated where they meet the introvert circular muscle, and their posterior ends extend toward the anterior region (pachycyclus) of the second trunk segment. These short muscles are the introvert circular muscle retractors (icmr) and extend internally along the region of the neck (Figures 4A, D, E, F; 5B; 6) (see below). When the introvert is everted, these muscles (icmr) are extended to reach their maximum length (Figure 6A). Inversely, when the introvert is retracted, the introvert circular muscle retractors contract to their minimum length (Figure 6C).

Musculature of the neck

The neck region contains a circular muscle (ncm) composed of three wide fibers. This neck circular muscle (ncm) is located internally to the placids of the neck, and is anchored distally to the soft cuticle of the interplacid areas (Additional file 2, Figures 4A-G; 5A-B). When the introvert is everted, the neck circular muscle is expanded, while it is constricted when the introvert is retracted (Figures 7A-B; 8A-F). It should be stressed that this neck circular muscle (ncm) has a fixed position in the adults of *Echinoderes* (compare position in Figure 4A, D, G) as a result of its anchoring points within the interplacid areas of the neck (Figure 7B). However, in contrast, the position of the introvert circular muscle (icm) is not fixed, and is repositioned along the anterior-posterior axis by eversion and retraction movements of the introvert (Figure 8A-F). Additionally, there are four to six fibers extending from longitudinal musculature of the trunk (see below) that attach within the neck region. These muscle fibers attach in between sets of ventrolateral placids within the interplacid region of the neck (Figures 4A, D, G; 7A-B).

Musculature of the trunk

The musculature of the trunk consists of several different muscle groups, including muscle fibers that span multiple segment margins, and distinct subsets of muscles that are clearly segmental in their orientation and attachment characteristics (Additional file 2, Figures 8A-F; 9A-D). Bilateral sets of individual muscle bands extend longitudinally along the anterior-posterior axis as continuous fibers that span more than one segment, and therefore do not follow a segmental pattern. These muscles include longitudinal retractors of both the introvert (irm) and pharynx (prm), and distinct bundles of fibers that extend from the neck circular muscles to segment 9 along ventrolateral sides of the trunk (Figures 8, 9). The ventrolateral muscles are distinguished here as lateral continuous fibers (lcf), which appear to attach at their anterior ends to the interplacid areas within the neck (Figure 7A, B) and the first segment (Figures 4A, D, G; 9C), and extend from there to putative attachment sites within consecutive posterior segments of the trunk; their final attachments are made by several fibers within segment 9 (Figures 8A, D, F; 9C). The number of fibers attaching within consecutive trunk segments decreases from anterior-to-posterior. Moreover, in some specimens the lateral continuous fibers (lcf) appear to overlap with diagonal segmental musculature within the first and second segments.

Segmentally arranged musculature is observed within trunk segments 1-10, and includes four distinct groups, in three orientations. Pairs of ventral and dorsal muscles are oriented longitudinally in each segment. There is one pair of ventral muscles (vm), and two pairs of dorsal muscles (dm) that are in subdorsal and laterodorsal positions within each segment (Figures 8C, E; 9 A-B, D). In some cases, dorsal muscle fibers may span more than one segment. In segments 1-8, there are pairs of diagonal muscle bands (dm) on left and right lateral sides of the body (Figures 8A-F;

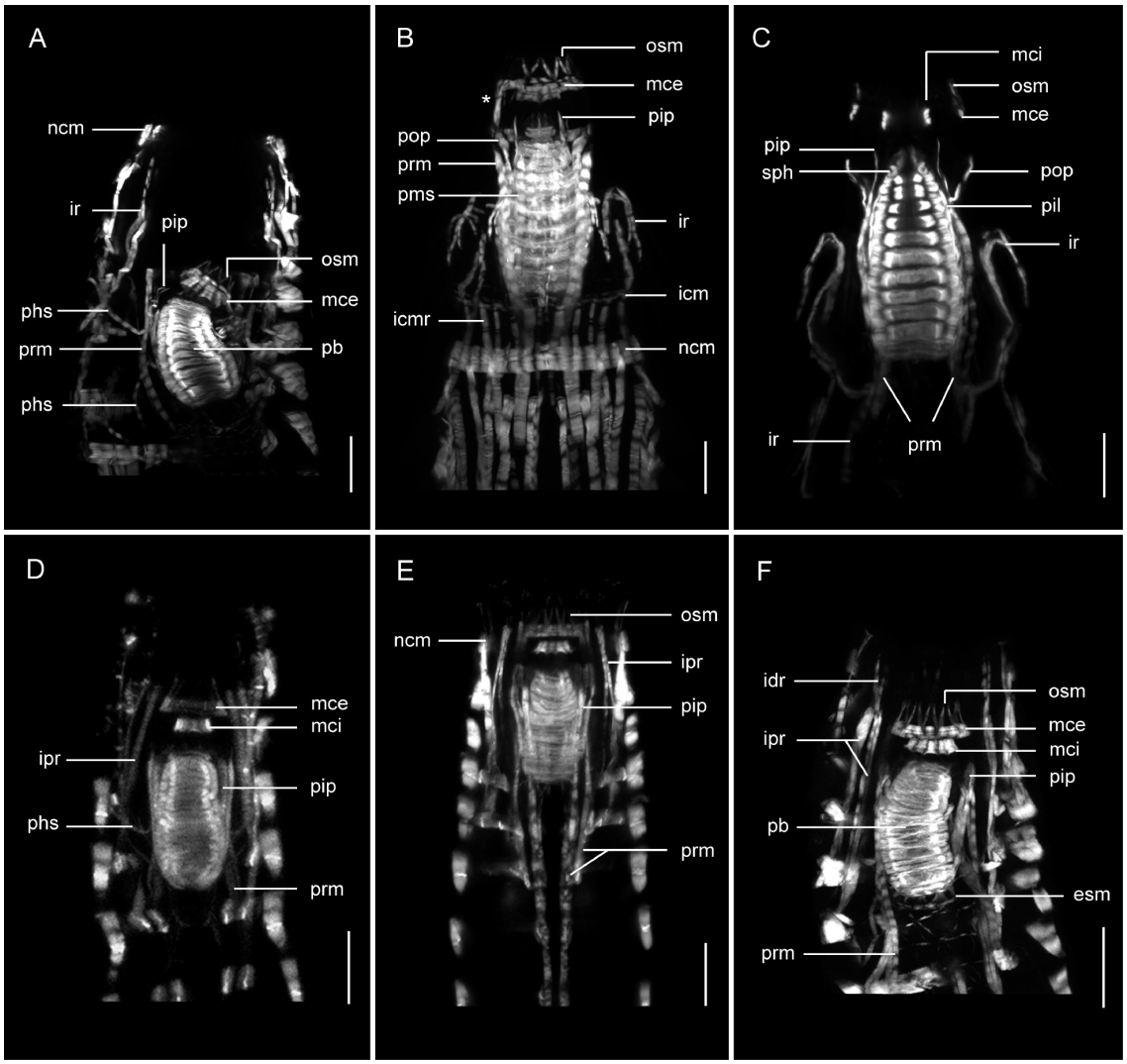

Figure 5 Musculature of the pharynx, introvert and mouth cone in *Echinoderes*. Confocal z-stack projections of phalloidin staining within the pharynx at different degrees of retraction. Ventral views, with anterior to the top in all images. **(A-C)** *Echinoderes dujardinii*. **(D-F)** *Echinoderes spinifurca.* In each micrograph, there are distinct muscle fibers connecting with, and enclosing, the pharyngeal bulb, including protractors, retractors, suspensors, and fibers of the pharynx sheath. An asterisk marks a muscle connecting the pharynx with the mouth cone external circular muscle. Abbreviations: esm, esophagus muscles; icm, introvert circular muscle; icmr, introvert circular muscle retractors; idr, introvert distal retractor; ipr, introvert proximal retractor; ir, introvert retractor; mce, mouth cone external circular muscle; mci, mouth cone internal circular muscle; ncm, neck circular muscle; osm, outer oral style muscle; pb, pharynx bulb; phs, pharynx suspensor; pip, pharynx internal protractor; pms, pharynx muscular sheath; pop, pharynx outer protractor; prm, pharynx retractor muscles; psm, pharynx sheath muscles; sph, sphincter. Scale bar, 20 µm.

9C). The fibers in each band appear to attach along anterolateral margins of the tergal (dorsal) pachycyclus, extend in an oblique or diagonal pattern, and converge medially to reach the pachycyclus of the following segment near each tergosternal plate junction (Figures 4A, D, G; 8C; 9A-C). The number of diagonal fibers within each segmental muscle band is variable among segments and species, with the first and last muscle bands of the series (segments 1, 2 and 8) typically having fewer fibers than observed within the other segments (Figure 9C). There are distinct pairs of dorsoventral muscles (dvm) in segments 3-10 (Figure 8A-D, F; 9A, B, D). Each dorsoventral muscle band is composed of two fibers. The fibers from each muscle of the pair insert on their respective left and right midventral sides of a segment, and extend dorsolaterally to symmetric attachment sites on the tergal cuticular plate in the middles of each segment (Figure 8A, C, D, F). Dorsoventral muscles (dvm) are oriented perpendicular to the anterior-posterior axis, and represent one of

the four pairs of segmental muscle bands, along with ventral (vm), dorsal (dm), and diagonal muscles (dm).

Additional musculature of the trunk is observed in segment 11, and is associated with lateral terminal spines (LTS), and penile spines (ps) of the males (Figure 10A-F). Each lateral terminal spine (LTS) has a pair of terminal spine levator muscles (tsl) that emerge laterally from the base of the spine and extend to the ventral side of the pachycyclus of segment 11 (Figures 8A-F; 10A-F). Another set of muscle fibers form the terminal spine depressor muscles (tsd), which extend from the opposite side of the lateral terminal spine base, and likely act as antagonists of the levator (tsl) muscles (Figure 10B, D). Short tergal extension muscles (tem) are associated with the basal part of each tergal extension, and they also seem to attach to the ventral side of the pachycyclus of segment 11, medial to the attachment of terminal spine musculature (Figures 8A-F; 10A, C, D). The lateral terminal accessory spines (LTAS) on segment 11 in the females of *Echinoderes* do not appear to be supplied with musculature (Figure 10A). In contrast, males have a pair of long, thin, dorsally curved muscles associated with their penile spines (Additional file 2, Figure 10B, C, E-F). A bilateral pair of penile muscles (pm) emerge close to the anterior ends of dorsal longitudinal muscles in segment 10, extend in a posterior direction, and then divide distally into three thin fibers that connect to the basal part of three penile spines on each side of segment 11 (Figure 10C, E, F). Therefore, there are two penile muscles (pm) that operate six penile spines (ps).

Gut musculature

The digestive system of *Echinoderes* is subregionalized into a foregut (mouth cone, pharynx, esophagus), midgut and hindgut. Distinct sets of muscles are integrated with each subregion. There is no evidence for segmentation of the musculature associated within or among different subregions of the digestive tract. The myoanatomy of the protrusible mouth cone, which contains the mouth and represents the beginning of the alimentary canal, is described above.

The pharynx of *Echinoderes* is a highly complex muscular organ (Figures 3, 4, 5, 9). The pharyngeal

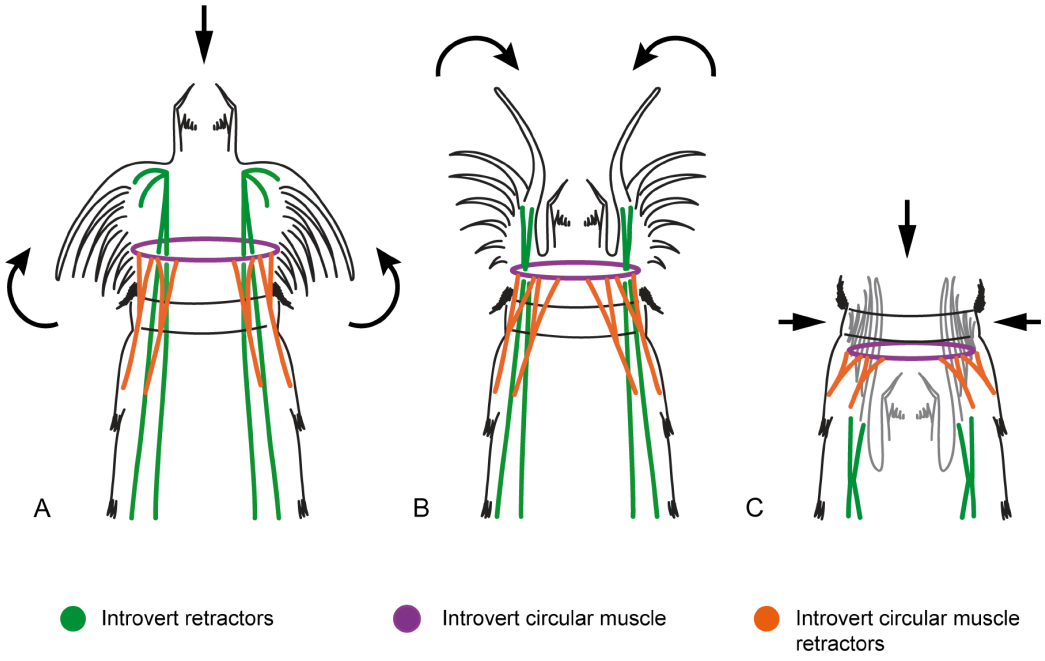

Figure 6 Schematic of muscle function during introvert retraction in *Echinoderes*. Transition from full eversion **(A)** to full retraction **(C)** of the introvert. Anterior is to the top. Only the muscle groups considered to be directly involved with introvert retraction are represented. Arrows indicate the directional movements of the introvert and scalids. **(A)** Introvert and mouth cone are everted. Introvert retractors, introvert circular muscle, and introvert circular muscle retractors are stretched. **(B)** Introvert is half-retracted, and the mouth cone is retracted. Introvert distal retractors are contracted, introvert proximal retractors are stretched, introvert circular muscle is contracted to a smaller diameter than the neck, and introvert circular muscle retractors are stretched. **(C)** Introvert and the mouth cone are fully retracted. Introvert retractors, introvert circular muscle, and introvert circular muscle retractors are now contracted and located within the trunk.

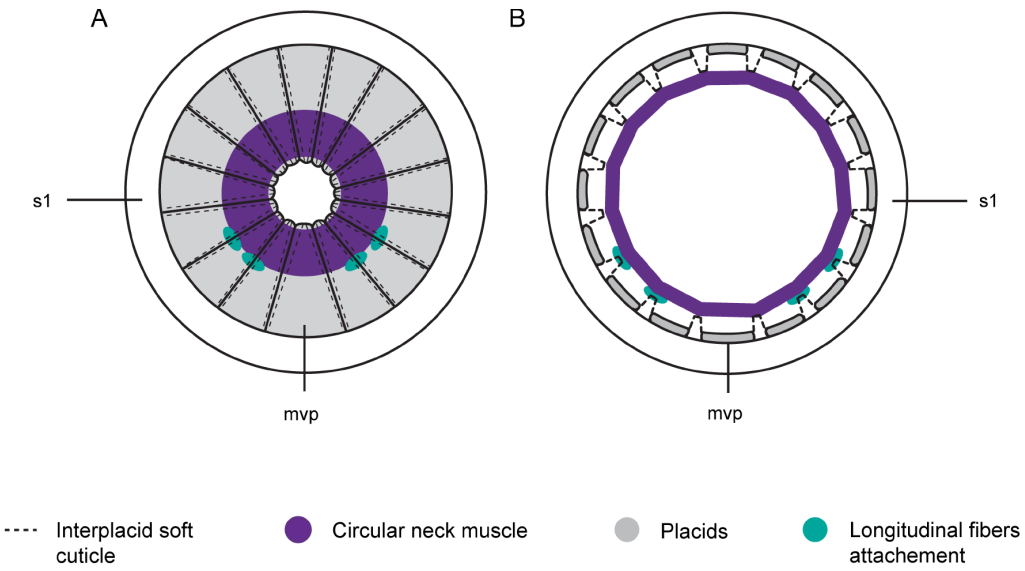

Figure 7 Schematic of the neck circular muscle and closing system in *Echinoderes*. Apical views, with ventral side to the bottom. **(A)** Neck circular muscle is contracted, and placids are rotated down toward the center of the neck; the neck is closed. **(B)** Neck circular muscle is stretched along interplacid attachment sites (dashed lines), and the placids are 'flipped open' along the anterior-posterior axis. The neck is open. Note the attachments of longitudinal fibers from the trunk to interplacid soft cuticle. Abbreviations: mvp, midventral placid; s1, segment 1.

bulb (pb) is a movable cylinder-shaped structure, and is composed of circular muscles (pcm) that alternate with radial muscles (pram) along the length of the bulb (Figures 3C-D; 4C, F, I; 5A-F; 9D). Ten bands of each type are counted, with circular bands being noticeably thinner than the radial bands (Figures 3C-D, 4I). A sphincter (sph) muscle is present at both anterior and posterior ends of the pharyngeal bulb (Figures 3C, 5C). The internal diameter of each sphincter is smaller than the internal diameters of circular and radial muscle bands. The pharynx is always positioned posterior to the buccal cavity; however, its position along the anterior-posterior axis correlates with movements of the mouth cone and introvert, and is therefore observed to vary among specimens according to the position in which they were preserved. Consequently, the pharynx is located inside the trunk when the head is retracted, and it reaches the mouth cone when the head is fully extended (Additional file 1, Figures 4C, F, I; 5A-F).

Around the pharyngeal bulb of *Echinoderes* sp., *E. hispanicus*, *E. horni* and *E. spinifurca* there are eight inner longitudinal muscles (pil). Each one of these muscles (pil) contains two fibers that extend posteriorly from the pharynx to the esophagus and midgut (Figures 2I, 3C). External to the inner longitudinal muscles (pil), there is a conspicuous tulip-shaped muscular sheath (pms) that encloses the pharyngeal bulb (Additional file 1, Figures 2D, 3D, 4I). This sheath is composed of eighteen pharynx retractor muscles (prm) arranged in pairs on their anterior end, which together form the nine tips of the tulip (Figures 2D, J, 3C). The eighteen, paired retractor muscles (prm) alternate positions with ten pharynx sheath muscles (psm) that do not extend posteriorly beyond the pharynx bulb (Figures 2L, 3C). Two of the ten pharynx sheath muscles occupy the middorsal position (Figure 2L). In addition, nine to ten inner protractor muscles (pip) contribute to the composition of the muscular sheath (pms) enclosing the pharynx. These inner protractor muscles (pip) alternate positions with the sheath muscles and most likely attach to the base of the mouth cone on their anterior ends, and to the base of the pharynx at their posterior ends (Figures 3C; 5D, F).

Four to six outer protractor muscles (pop) appear to attach to the posteriormost end of the pharynx at midventral, midlateral, and laterodorsal positions. These outer protractors (pop) extend from the base of the pharynx toward the head where they likely attach to the body wall area between the introvert and the mouth cone (Figure 3C). These protractors appear J-shaped when the pharynx extends toward the animal's anterior end, surpassing their insertion point (Figures 3C; 4F, I).

In *E. dujardinii* there are also eight inner longitu-

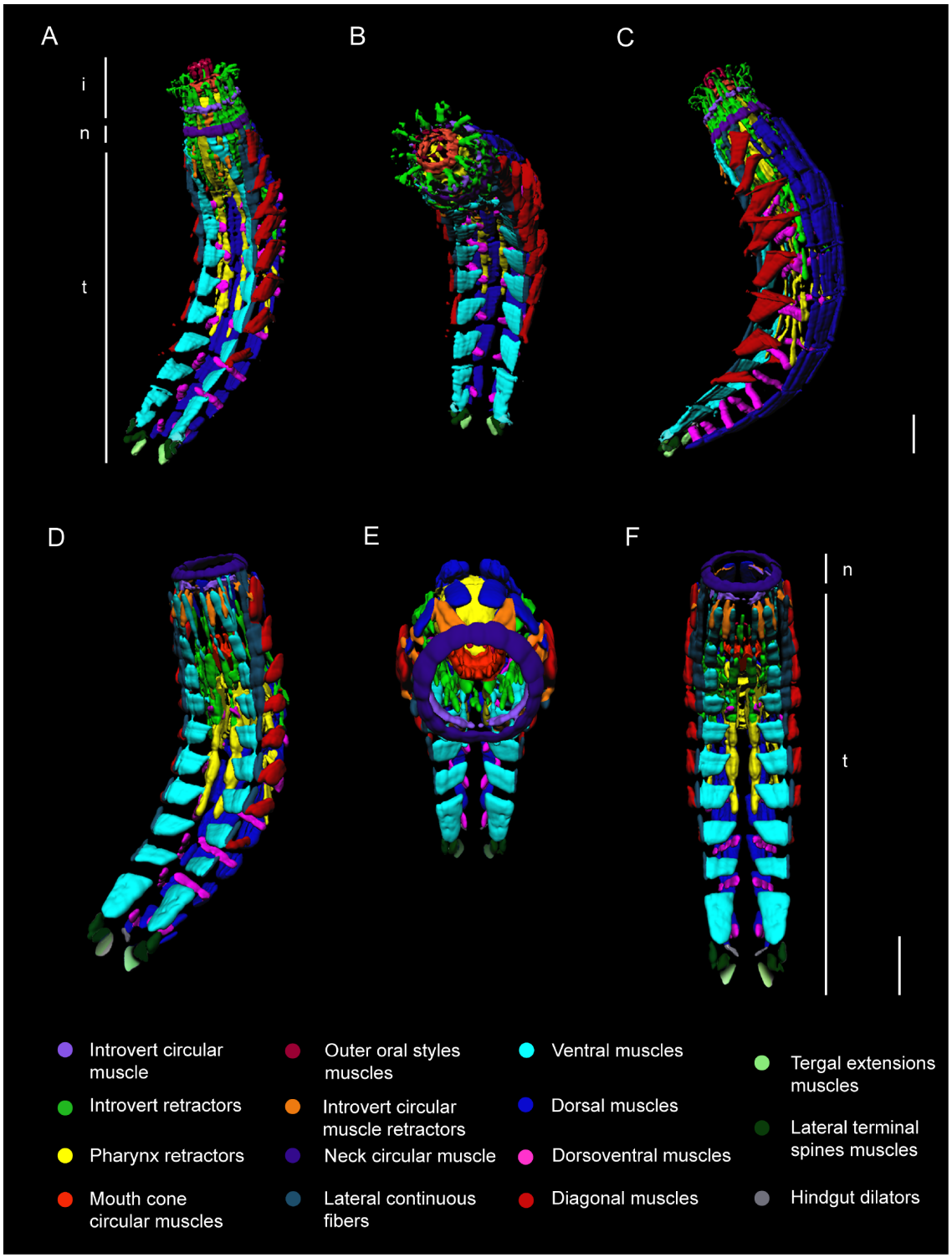

Figure 8 Three-dimensional reconstruction of myoanatomy in *Echinoderes horni*. **(A-C)** Single specimen with the introvert fully everted. **(D-F)** Single specimen with the introvert fully retracted. Anterior to the top in all images. Notable musculature: **(A)** latero-ventral view: outer oral style muscles, neck circular muscle, segmented trunk muscles; **(B)** lateroventral, with introvert in apical view: mouth cone circular muscles, introvert retractors; **(C)** lateral view: diagonal muscles, dorsal muscles, dorsoventral muscles; **(D)** latero-ventral view: ventral muscles, lateral continuous fibers, terminal spine muscles; **(E)** apical-ventral view showing introvert and mouth cone within the trunk: neck circular muscle, introvert circular muscle; **(F)** ventral view: introvert circular muscle retractors, ventral muscles, pharynx retractors, terminal spine muscles, tergal extension muscles, hindgut dilators. Abbreviations: i, introvert; n, neck; t, trunk. Scale bar, 30 µm.

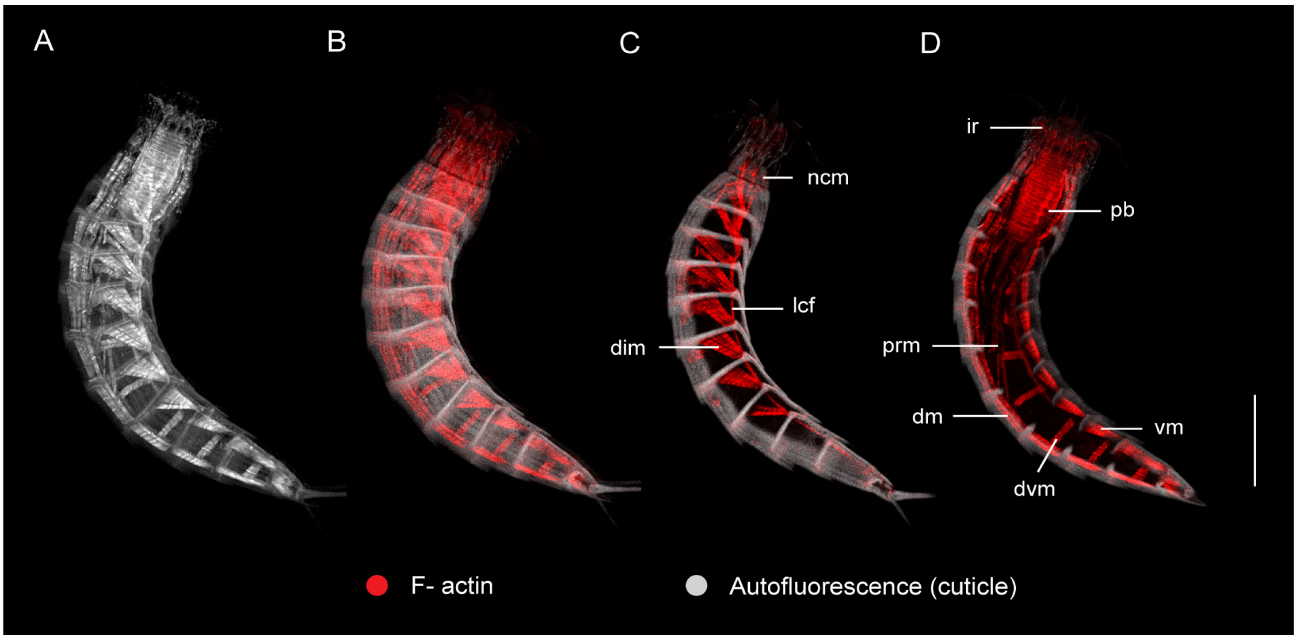

Figure 9 Musculature within the segmented trunk region of *Echinoderes horni*. Confocal z-stack projections of phalloidin staining (muscle) and autofluorescence (cuticle) in a single specimen. Lateral views with anterior to the top and ventral to the right side. **(A-B)** maximum z-stack projections of muscle in grayscale (A) and red (B). **(C)** z-stack of optical slices on the right side of the medial plane. **(D)** z-stack of optical slices through the medial plane at the level of the pharynx. Ventral, dorsal, dorsoventral and diagonal muscles are segmented. Continuous fibers span multiple segments on dorsal and ventrolateral sides of the trunk, and within the trunk as pharynx retractors. Abbreviations: dim, diagonal muscles; dm, dorsal muscles; dvm, dorsoventral muscles; ir, introvert retractors; lcf, longitudinal continuous fibers; ncm, neck circular muscle; prm, pharynx retractor muscle; vm, ventral muscles. Scale bar, µm.

dinal muscles (pil) surrounding the pharyngeal bulb as described above, although their paired nature cannot be assessed (Figure 5C). Nine inner protractor muscles (pip) with a flame-like shape surround the pharyngeal bulb, and extend well beyond the bulb's anterior end to attachment sites at the basal end of the mouth cone (Figure 5B-C). In some specimens, one or two of these retractors (pip) appear to be connected with the internal mouth cone ring. The muscular sheath that surrounds the pharynx (pms) of *E. dujardinii* is composed of the inner protractor muscles (pip) that alternate with ten or more pharynx retractors (prm), and ten sheath muscles (psm) (Figure 5B). An undetermined number (2-4) of outer protractors (pop) are present as well, which attach to the base of the mouth cone, and may connect with the external circular muscle (mce) of the mouth cone (Figure 5B asterisk). The general appearance of the sheath in *E. dujardinii* resembles a 'basket' around the pharynx. This muscular basket-like structure can be found more or less contracted depending on the position of the pharynx, as observed in a more contracted state when the pharynx is either extended to the mouth cone, or retracted away from the mouth cone.

An additional group of thin longitudinal muscle fibers are observed in all five species. These fibers extend from the middle of the pharynx to segments 3 and 4. They appear to be relaxed when the pharynx is retracted, and not readily visible when the pharynx is extended. These particular muscles have been termed pharynx suspensors (phs) due to their position, morphology and possible function (Figures 3C, 5A, D).

Posterior to the pharyngeal bulb in each species, pharynx retractors (prm) are arranged in four groups, with two oriented in lateroventral positions, and two in laterodorsal positions (Figures 3C, 5E, F). These pharynx retractors (prm) extend into the trunk toward ventromedial and laterodorsal attachment sites within segments 5-8 (Figures 4C, F; 5C, D, F; 8D).

The musculature of the midgut is composed of sixteen inner longitudinal muscles (mil), and sixteen or more thin, outer circular muscles. These filamentous muscle fibers are arranged in an orthogonal grid-like (gg) architecture encircling the midgut intestine (Figure 3D). At the level of the esophagus, the inner longitudinal muscles of the midgut (mil) bifurcate from the eight pairs of inner longitudinal muscles of the

pharynx (pil), and extend posteriorly to surround the intestine and the hindgut (Figure 3D). Between segments 10 and 11, two pairs of short transverse muscle fibers extend from laterodorsal muscles and appear to tether the hindgut laterally. These fibers were designated as hindgut dilator (hd) muscles (Figures 3D, 7F, 9A-B).

Discurssion

Comparative myoanatomy within Echinoderes

Among the five species of *Echinoderes* we examined, there is notable variation in external morphology, although it is primarily limited to differences in the number, length, and presence or absence of small cu-

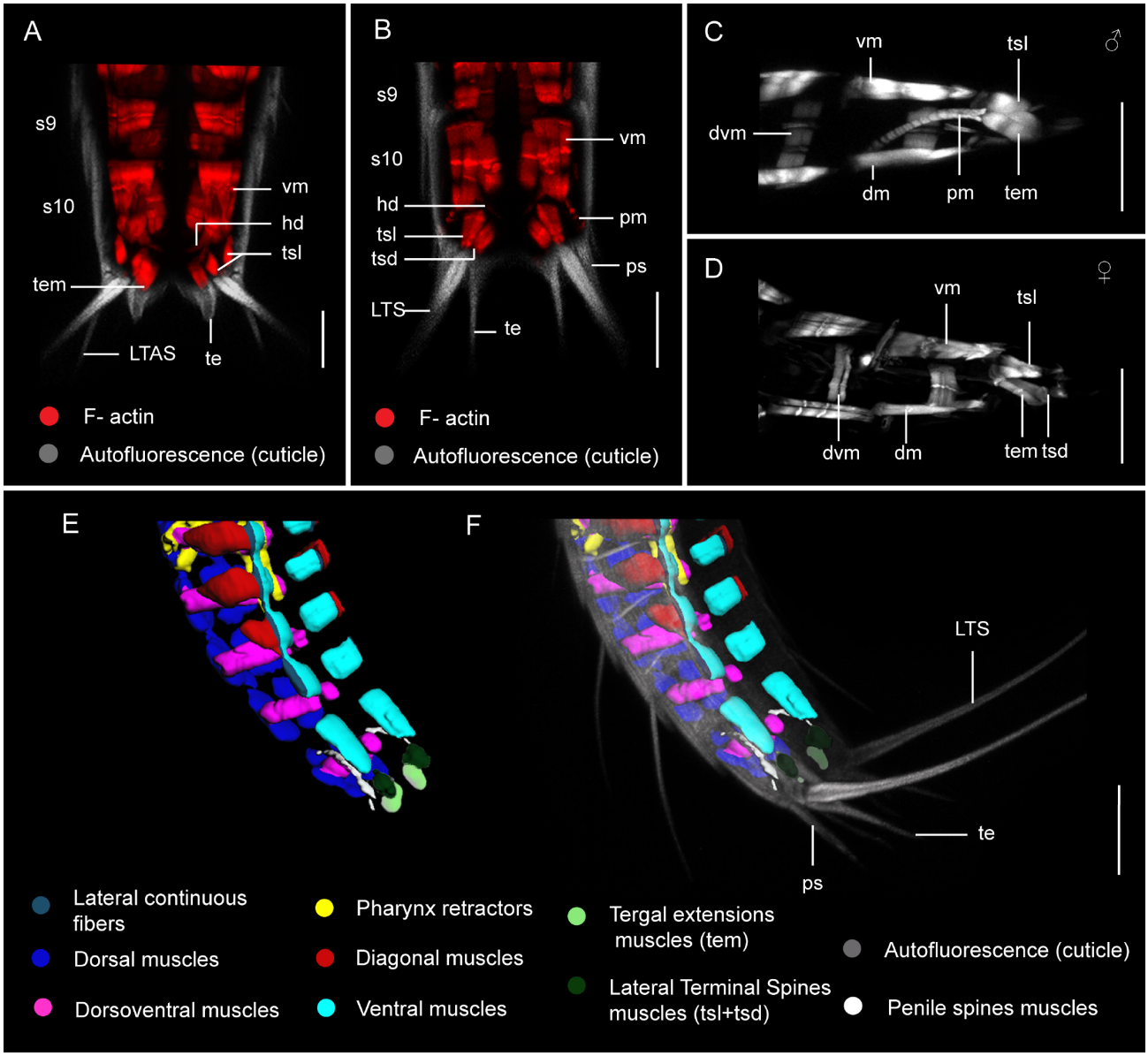

Figure 10 Musculature of the posterior trunk region and spines in *Echinoderes*.
(A-B) Confocal z-stack projections of phalloidin staining (muscle, red) and autofluorescence (cuticle, grayscale) in segments 9-11 of *Echinoderes horni* (A) and *Echinoderes spinifurca* (B) in ventral view, with posterior to the bottom. **(C-D)** Confocal z-stack projections of phalloidin staining in the posterior end of *Echinoderes horni* (C, male) and *Echinoderes spinifurca* (D, female) in lateral view, with posterior to the right side. **(E-F)** 3D reconstruction of myoanatomy in segments 7-11 of *Echinoderes spinifurca* in lateroventral view with posterior to the right side. Autofluorescence of the cuticle is included in (F). Abbreviations: dm, dorsal muscle; dvm, dorsoventral muscle; hd, hindgut dilator; LTAS, lateral terminal accessory spine; LTS, lateral terminal spine; pm, penile spine muscle; ps, penile spine; s, segment; te, tergal extension; tem, tergal extensions muscle; tsd, terminal spine depressor; tsl, terminal spine levator; vm, ventral muscle. Digits in (A) and (B) refer to the segment number. Scale bar: 20 µm.

ticular structures (Table 1). Variations in the external morphology of those species are reflected by relatively minor differences in the internal morphology of their musculature. Most of this variation includes species-specific differences in the head region, such as the shape, length and number of retractor muscles of the introvert and pharynx, as well as the composition of the pharyngeal sheath. Musculature within regions of the neck and trunk is basically similar among species. Overall, comparative myoanatomy within *Echinoderes* is remarkably conserved, and includes the following set of common characters: (Head) two circular muscles and nine pairs of oral style muscles in the mouth cone; ten longitudinal retractors, one circular muscle, and fourteen circular muscle retractors in the introvert; (Neck) one circular muscle; (Trunk) continuous intersegmental fibers among segments 1-9, and segmental pairs of ventral, diagonal, dorsoventral and dorsal muscles within segments 1-10 of the trunk; three pairs of terminal spine muscles and one pair of penile spine muscles in segment 11; (Gut) a pharyngeal bulb with ten radial and ten circular muscle bands enclosed in a muscular sheath, including protractor and retractor muscle fibers; an orthogonal grid of midgut muscle fibers, and lateral pairs of hindgut dilator muscles.

Prior to our multi-species investigation with the aid of a confocal laser scanning microscope (CLSM), the myoanatomy of *Echinoderes* was understudied. Microscopical descriptions for the purpose of taxonomic identification or anatomy were conducted with compound light (LM) or transmission electron (TEM) microscopes, and they presented limited coverage of the musculature. From those studies, data are available for a few species, including *Echinoderes aquilonius*, *E. capitatus* and *E. dujardinii* [1, 33, 41, 42]. Upon review, it appears that the anatomy of neck circular muscles, and the number and arrangement of longitudinal, diagonal and dorsoventral muscles in the trunk, is shared among those species. Those results are in agreement with our study. However, there are noticeable differences between the results of previous work and our study regarding the arrangement of muscles in the head, the first trunk segments, and the gut. These particular regions include not only locations in the kinorhynch body plan where multiple types of muscles overlap, but also the most intricate and difficult myoanatomy to interpret, which may introduce both real and artificial discrepancies among results. Moreover, it is also likely that different *Echinoderes* species from separate studies were examined with different levels of focus and attention to detail. Thus, it is necessary to compare our results with different species from separate studies in order to resolve several issues (Table 2).

Kristensen and Higgins [1] described retractor muscles in the introvert of *E. aquilonius* that consisted of two sets, one composed of sixteen outer retractors, and the other set with twelve inner retractors. Their descriptions differ from what we observe in *Echinoderes*. In *E. aquilonius*, the sixteen outer retractors appear to extend from segment 9, pass through the forebrain, and attach to the anteriormost end of the introvert [1]. We identified ten introvert retractors in each of five species from approximately one hundred specimens. These ten retractors also extend through the forebrain, however, they attach at the level of the first row of spinoscalids and alternate positions with them. Kristensen and Higgins [1] may have misidentified some protractors of the pharynx as introvert retractors, leading to the wrong assignment and a higher total count. Despite these differences in number and arrangement, our introvert retractors may be the "outer retractors" described by Kristensen and Higgins [1]. We also identified 18-20 pharynx retractors, which may correspond to the "inner retractors" of *E. aquilonius*, although our count differs again from the twelve described [1]. These and other differences likely stem from our application of fluorescent markers and CLSM, which enabled staining and visualization of complete sets of muscles in multiple focal planes. Additionally, we labeled cellular structures of the brain, introvert and pharynx for contextual landmarks, and the ability to discriminate between distinct muscle fibers within highly complex regions of kinorhynch myoanatomy. As a result, we reevaluated previous ambiguities, such as *inner* and *outer* retractors [1], in order to recount and reassign organ-specific fibers and muscle groups (e.g. introvert distal retractors, pharynx outer protractors).

The number and arrangement of mouth cone muscles reported herein appears different from what was found in *E. capitatus* [33], where three circular muscles were described, without reference to any longitudinal fibers. In contrast, we clearly identified two circular muscles, and nine pairs of short longitudinal muscles in each of the five echinoderid species. Nebelsick [33] may have easily overlooked these longitudinal muscles due to their small size and position within

the mouth cone. Regarding the number of mouth cone circular muscles, Nebelsick [33] shows three individual muscles in *E. capitatus* (see Figure 1therein), however we suggest that based on their positions they are actually two circular muscles which correlate with what we found in our study.

Circular muscles of the neck are commonly identified in studies of *Echinoderes*. Zelinka [41] described a single muscle in the neck region, whereas Remane [42] described five rings in *E. dujardinii.* Our results corroborate the observations of Zelinka [41], and suggest that Remane [42] could have misidentified circular muscles from the introvert and mouth cone as additional neck musculature. Kristensen and Higgins ([1], see Figure 27 therein) presented a detail of circular muscle attachment in the neck of *E. aquilonius*, although it was not described. We identified a similar arrangement between the neck circular muscle and its proximity to the interplacid areas (Figure 7B). This particular attachment site, where the neck circular muscle is oriented along the anterior end of the placids, and attached to softer interplacid cuticle, was confirmed by additional evidence (R. M. Kristensen, personal communication).

The presence of continuous longitudinal fibers in the trunk of *Echinoderes* is also supported by TEM images of *Echinoderes cantabricus*, but was not discussed by Gª Ordóñez et al. [34]. Accordingly, Remane [42] described a similar pattern in *E. dujardinii* as the splitting of longitudinal muscles in the anterior end of the trunk. This 'splitting' of muscles described by Remane [42] most likely corresponds to the longitudinal continuous fibers that fan out within the trunk of *Echinoderes* species investigated here. The sixteen longitudinal midgut muscles of the orthogonal gut grid observed in *Echinoderes* species in our study also support previous observations by Kristensen and Higgins [1] who described 12-16 longitudinal muscles in the trunk of *E. aquilonius*.

Comparative myoanatomy of Kinorhyncha

Within Kinorhyncha, there have been TEM studies of internal morphology and ultrastructure, with details of myoanatomy in several genera: *Zelinkaderes floridensis* [30], *Pycnophyes dentatus* [30], *Pycnophyes flaveolatus* [38], *Pycnophyes greenlandicus* [1], and *Pycnophyes kielensis* [30, 5]. Previous descriptions of musculature by confocal microscopy have also been presented, although they were limited to one cyclorhagid species, *Antygomonas* sp. [26], and one homalorhagid species, *P. kielensis* [27, 5]. Thus far, most of the attention has been on longitudinal and segmental musculature of the trunk, with only one description of myoanatomy in the head and neck [26]. Unfortunately, all of the specimens in that study had their introvert fully or partially retracted, precluding detailed resolution of anterior musculature, such as important differences between the anterior radial closing system of Echinoderidae and the bilateral closing system of species within Antygomonidae. Yet, when considering all of the available information, *Antygomonas* sp. [26] is the most appropriate model for direct comparison with *Echinoderes*.

We found eighteen outer oral style muscles in each species of *Echinoderes* studied here, representing one pair of muscles for each outer oral style. Müller and Schmidt-Rhaesa [26] reported sixteen outer oral style muscles, which would indicate that one of the nine oral styles of *Antygomonas* sp. does not have a pair of muscles. Additionally, we did not find any of the eight basal mouth cone muscles described in *Antygomonas* sp. [26; see Figure 4A therein]. These short, single mouth cone muscles may be exclusive to Antygomonidae, or perhaps they are attachment sites for the sixteen mouth cone muscles. We did identify 14-16 short introvert circular muscle retractors (icmr) in each species of *Echinoderes*, which are most likely comparable to these singular fibers that "stretch toward the first circular ring" in the neck region of *Antygomonas* sp. [26]. Although they were not characterized as circular muscle retractors, we consider them as such because they appear to extend from the introvert circular muscle toward the body wall in the first segment, which is comparable to the condition of 'icmr' fibers in *Echinoderes*. We have clearly identified fourteen introvert circular muscle retractors in *Antygomonas paulae* (unpublished observations, Herranz and Boyle). The outer retractors (or) of *Antygomonas* sp. [23] should also be considered comparable to the introvert distal retractors in *Echinoderes*. Müller and Schmidt-Rhaesa [26] observed two mouth cone circular muscles, as well as the introvert and neck circular muscles within *Antygomonas* sp., which match our observations in *Echinoderes*. However, we disagree on the proposed functions of the introvert and neck circular muscles. Whereas both muscles are ultimately involved in introvert retraction

Table 2. Comparative data on the myoanatomy of Kinorhyncha from this study and available primary literature (references included).

Species	***Echinoderes aquilonius***	***Echinoderes capitatus***	***Echinoderes dujardinii***	***Echinoderes* sp.** ***E. dujardinii*** ***E. hispanicus*** ***E. horni*** ***E. spinifurca***	***Antygomonas* sp.**	***Zelinkaderes floridensis***	***Pycnophyes dentatus***	***Pycnophyes flaveolatus***	***Pycnophyes kielensis***
Data source	TEM	TEM	LM	CLSM	CLSM	TEM	TEM	TEM	TEM, CLSM
Mouth cone	-	3 circular muscles	-	18 longitudinal, 2 circular muscles	16 longitudinal, 8 short basal and 2 circular muscles	18 longitudinal muscles	2circular cell basal to oos, 7 circular muscle cells basal to ios	-	1circular muscle cell basal to oos, 7 circular muscle cells basal to ios
Introvert	16 outer retractors 12 inner retractors	10 retractors reaching S.6	-	10 retractors reaching S.6, Introvert circular muscle	16 outer retractors reaching S.8	-	10 head retractors	-	10 head retractors
Neck	-	-	1- 5 circular muscles	1circular muscle	2 circular muscles: inner outer	-	-	-	-
Trunk	-	-	Longitudinal: dorsal, ventral, and additional continuous fibers, Diagonal: 8 pairs Dorsoventral: S.3-11	Longitudinal: Ventromedial+ Dorsolateral and additional continuous fibers, Diagonal: 8 pairs Dorsoventral: S.3-11	Longitudinal: Ventrolateral+ Dorsolateral Diagonal: 9 pairs Dorsoventral: S.1-11	-	-	-	-

Spines	-	-	-	3 pairs LTS+ 1 pair TE muscles 1pair penile spines muscles	Midterminal spine muscles triangle- shaped	-	-	-	-
Pharynx	Circular lumen	-	-	Circular lumen, 10 radial+10 circular fibers	Circular lumen, 14-15 circular fibers alternate with inhomogeneous structures, pre- and postpharyngeal sphincters 10 protractors, Pharynx sheath present	Circular lumen, 15 radial +15 circular fibers, 2 prepharyngeal 1postpharyngeal sphincters	Triradiate lumen 13 radial+14 circular muscle cell 1postpharyngeal sphincter	Triradiate lumen Circular and radial muscular fibers 1postpharyngeal sphincter	Triradiate lumen 13 radial+14 circular muscle cell 1postpharyngeal sphincter
Midgut	12-16 longitudinal muscles	-	-	Muscular grid: Inner longitudinal (16)+ outer circular (+16)	Muscular Grid: Inner longitudinal + outer circular	Muscular Grid: Inner longitudinal + outer circular	Muscular Grid: inner longitudinal + outer circular	-	Muscular Grid: inner longitudinal + outer circular
Hindgut	-	-	-	1-2 pairs short dilator muscles	1 dilator muscle	6 dilators: 2 caudal + 2 dorsal 1 frontal 1 circular	4 dilators: 2 caudal + 2dorsal; 2dorsal + 1 ventral transversal muscle cell	-	4 dilators: 2 caudal + 2dorsal; 2dorsal + 1 ventral transversal muscle cell
Reference	[1]	[33]	[41, 42]	This study	[26]	[30]	[30]	[38]	[5, 27,30]

Myoanatomical features observed using transmission electron microscopy (TEM), light microscopy (LM) or confocal laser scanning microscopy (CLSM). Abbreviations: LTAS, lateral terminal accessory spines; LTS, lateral terminal spines; S, segment; (-) data not available.

and closure of the anterior trunk in *Echinoderes*, they are most likely independent systems, where introvert retraction is dynamic and often partial without closing off the trunk body. Contrary to this, Müller and Schmidt-Rhaesa [26] suggested that both muscles primarily control the closing system (neck) of *Antygomonas* sp. However, each of their respective closing systems exhibits a distinct morphology and thus requires a more thorough investigation.

Within the trunk region, intersegmental and segmental muscle bands most likely occur in all kinorhynchs. However, diagonal muscles have been described almost exclusively for cyclorhagids [41, 42], with only one report of diagonal bands in a homalorhagid species, *Pycnophyes calmani* [47]. The diagonal muscles of *Echinoderes* show a clear segmental pattern along the trunk, while in *Antygomonas* sp., each of the first three diagonal muscles span two segments [26]. These differences could arise from variation in musculature of the neck and first trunk segments associated with radial vs. bilateral closing systems. The dorsoventral muscles are the only segmental muscles of the trunk that do not attach to pachycycli, and therefore they do leave conspicuous cuticular scars [1, 26, 41, 42]. These muscles are absent in the first and second ring-like segments of *Echinoderes*. Other genera within Echinoderidae, and Kinorhyncha, vary as to whether segment 2 is composed of one or two sternal plates, which may correlate with changes in the arrangement and attachment of musculature in the first two segments. As previously mentioned, detailed comparative studies of kinorhynch closing systems are needed to address such questions.

Myoanatomy of the pharyngeal bulb has been described in several genera and species [1, 26, 30]. Within the pharynx, there are notable differences in the shape of the lumen, and the number of circular and radial muscle fibers. In Cyclorhagida, including *Echinoderes*, the shape of the pharyngeal lumen is primarily circular, while in Homalorhagida the lumen exhibits a triradiate, or inverted Y-shaped, configuration [17, 18]. Since triradiate pharynges are most likely homologous in Ecdysozoa, this would suggest that Homalorhagids exhibit the primitive kinorhynch gut architecture [17]. Yet we remain cautious, as the first molecular phylogeny of kinorhynchs did not recover monophyly for either Cyclorhagida or Homalorhagida [48], and other evidence suggests that a triradiate pharynx "cannot be ancestral in the cycloneuralians" and likely evolved in parallel multiple times [18]. Regarding variation in the number of alternating circular and radial fibers among genera, there are fifteen in *Zelinkaderes*, thirteen-to-fourteen in *Pycnophyes* and fourteen-to-fifteen circular fibers alternating with inhomogeneous structures in *Antygomonas* [26, 30, 32] (Table 2). Our results show ten circular and ten radial fibers in *Echinoderes*. This arrangement appears highly conserved among species, and may become an important diagnostic character for the genus. It is not known whether this arrangement of pharyngeal muscle fibers is shared within Echinoderidae.

Musculature associated with terminal spines has been reported in *Pycnophyes kielensis* [27], and a strong, paired muscle was described at the base of the midterminal spine in *Antygomonas* sp. [26]. Although Müller and Schmidt-Rhaesa [26] did not state it, their data also suggest there could be muscles in the bases of lateral terminal spines in *Antygomonas* sp. Additional kinorhynch genera with midterminal and lateral terminal spines include *Centroderes, Zelinkaderes* and *Semnoderes*, however spine musculature has not yet been investigated within or among these genera. The five species of *Echinoderes* in our study share a similar arrangement: muscles are distinctly associated with lateral terminal spines, but not with lateral terminal accessory spines, which are rigid and immobile in live animals. The presence of penile spines in male kinorhynchs is shared by *Cephalorhyncha*, *Dracoderes, Echinoderes*, *Fissuroderes*, *Meristoderes and Pycnophyes*. Of those genera Myoanatomical data are only available for species of *Echinoderes* and *Pycnophyes*, and until now, penile-spine musculature has not been identified in either genus. Our results show conspicuous muscles associated with penile spines in all five *Echinoderes* species, and we predict that similar muscles will be identified in species from each of the genera where penile spines are present.

Interpretations of functional myoanatomy in Echinoderes

With the exception of smooth muscles of the intestine, the sarcomeres of kinorhynch myofibrils have a predominantly cross-striated arrangement [1, 26, 27]. This enables rapid muscle contraction associated with feeding and locomotion [17]. Both feeding and some forward locomotion behaviors are produced by ever-

sion and retraction of the introvert, which is characterized by complex myoanatomy within head and neck regions (Additional file 3; [1]). Not only are well-developed circular and retractor muscles of the head and neck involved in movements of the introvert, but they also exemplify the unsegmented radial symmetry of the mouth cone, introvert and cuticular ring of placids, which support previous suggestions that there are no true segments in the head or neck region of kinorhynchs [1, 13, 49, 50].

Here, we propose a model for retraction of the introvert by combined action of three sets of muscles in *Echinoderes*: introvert retractors, introvert circular muscles, and introvert circular muscle retractors (Figure 6). When the introvert is everted, introvert retractors, circular muscles (icm), and circular muscle retractors (icmr) are relaxed and stretched (Figure 6A). Ten introvert retractor muscles are attached at the level of the primary spinoscalids and alternate with them in a radial arrangement. During contraction, introvert retractors cause the primary spinoscalids to fold inward, followed by the remaining scalids (Figure 6B). Synchronous contraction of introvert retractors and the relaxation of dorsoventral muscles within the trunk [1], combine to initiate retraction of the introvert. Musculature is absent within primary and other scalids, which implies that scalids cannot move independently from each other, and thus move passively during relocation of the head. The introvert circular muscle (icm) then contracts, reducing the volume and diameter of the introvert, enabling it to pass through the neck. At the same time, the introvert circular muscle retractors (icmr) contract, which supplements the introvert retractors, leading to retraction of the introvert (Figure 6C).

Dorsoventral muscles of the trunk play an important role during extension of the introvert. The contraction of dorsoventral muscle fibers causes an increase of internal pressure within the reduced body cavity, acting as a hydrostatic skeleton to extend the introvert [22, 17]. Once the introvert is everted, the mouth cone protrudes. Internal hydrostatic pressure within the body cavity may not be enough to fully extend the mouth cone. However, the mouth cone has an intimate positional relationship with the pharynx. When the pharyngeal protractor muscles contract, the pharynx moves in an anterior direction pushing the mouth cone outward (see below). When the pharynx is retracted toward the trunk by contraction of pharyngeal retractor muscles, the mouth cone is also retracted.

Typical food items found within the gut of kinorhynchs include bacteria, diatoms, algae and detritus [32, 33, 41, 51]. The articulated outer oral styles may be used as ‘forceps’ for grasping, manipulating, and ingesting those and other food items [54]. In order to feed this way, muscles at the base of the outer oral styles (osm) are most likely antagonistic with an external circular muscle (mce) of the mouth cone (Figure 3A-B). When the paired, longitudinal oral style muscles contract, the external circular muscle relaxes and the oral styles are straightened and extended (Figure 3A). Conversely, when the external circular muscle contracts, the paired oral style muscles stretch and the outer oral styles bend (Figure 3B). The radial distribution of oral style muscles, as well as introvert retractors, indicate a correlated function. As previously suggested, this combination of longitudinal and circular muscles may enable limited motion of the outer oral styles [30]. Their main function could be to sense and respond to external stimuli, which is in agreement with TEM studies that show a sensory cell at the base of each outer style [1, 29, 33]. However, both sensory and grasping functions may be combined. It would seem reasonable that the animal must detect and discriminate what is ingested. The inner oral styles are not articulated, and show only a circular muscle around their base. This may function to selectively open the mouth in the presence of food particles [1, 30, 52]. Furthermore, ciliated receptor cells and terminal pores have been confirmed in each of the inner oral styles, suggesting there may also be a sensorial feeding-related function for these structures [1, 35].

The neck acts as an anterior closing apparatus of the body by synchronous movements of alternate hard (placid) and soft (interplacid) cuticular elements [41, 42]. The radial symmetry of this ‘closing system’ is shared by all genera within Echinoderidae. In *Echinoderes,* the neck circular muscle attaches to soft interplacid cuticle lining the distal perimeter of the neck region (R.M Kristensen personal communication). When the introvert is retracted, the placids rotate toward the center of the neck on hinge-like articulations between the placids and the first trunk segment (Figure 7A, 1H). When the introvert is everted, the neck circular muscle is relaxed to its widest diameter along interplacid attachment sites. In this relaxed position, the hard placids

are 'flipped open' and aligned with the anterior-posterior axis (Figure 7B). Rotation of the placids is coordinated with contraction of longitudinal fibers (lcf) that are attached to interplacid sites of the neck (Figure 7B). When the introvert is completely withdrawn, the circular muscle is contracted to its smallest diameter, soft interplacid cuticle is pulled toward the center of the neck, and the placids become tightly juxtaposed to close off the anterior of the trunk (Figures 1H, 7A). Consequently, the neck and introvert are functionally linked and interdependent [1, 50]; however, while circular muscles of the mouth cone and introvert become repositioned along the anterior-posterior axis, the neck circular muscle maintains a fixed position.

The trunk has paired sets of longitudinal, dorsoventral and diagonal muscles that enable these animals to perform a range of movements. In *Echinoderes*, the relaxed body plan exhibits a curvature of the dorsoventral axis along the trunk (Figure 1A-E). Segmented dorsal and ventral longitudinal muscles may act as antagonists to produce this curvature. And paired sets of continuous fibers that span several segments likely contribute to the high degree of trunk flexibility observed in living specimens (Additional file 3). Pairs of dorsoventral muscle bands join tergal (dorsal) and sternal (ventral) plates in segments 3-10. These muscles are thought to be derived from circular muscles [1, 53]. However circular muscles are reduced or absent in the trunk of kinorhynchs, and a reasonable hypothesis for the origin of dorsoventral musculature is lacking. Yet, as with other muscle types, dorsoventral muscle bands are integral to kinorhynch locomotion and feeding. The contractions of dorsoventral fibers pull each plate toward the center of the body, which increases the internal pressure of the trunk and contributes to eversion of the introvert. To our knowledge, contraction of dorsoventral musculature has not been characterized in *Echinoderes*, or Kinorhyncha. We suspect that during introvert eversion, contraction of these muscles would proceed sequentially from posterior to anterior, analogous to peristaltic movements in soft-bodied invertebrates. Synchronous contraction may not be as effective, impeding anterior movement of the pharynx and mouth cone. Diagonal muscles are only present from segments 1-8, and contribute to lateral movements of the animal within that trunk region [41, 42]. Because they are not present in segments 9-11, the posterior trunk is less flexible, which may confer an unrecognized functional stability associated with sexual reproduction.

We did not detect musculature within dorsal and lateroventral spines. Müller and Schmidt-Rhaesa [26] described small, triangular paradorsal muscles at the base of middorsal spines in *Antygomonas* sp. that could be responsible for the movements of those spines. However, posterior studies revealed that actin filaments in circumciliary microvilli of paradorsal sensory spots were misinterpreted as muscles [17, 32]. Among species of *Echinoderes*, we observe a similar pattern of 'triangular' labeling in trunk positions corresponding to sensory spots, which appears to support the alternative explanation for the presence of actin filaments in microvilli. Musculature associated with lateral terminal spines, tergal extensions and penile spines appear to be specializations of the ventral and dorsal longitudinal musculature [26]. It is not known whether segmented ventral or dorsal muscles act simultaneously or independently with spine movements. Lateral terminal spines are directly controlled by antagonistic pairs of short muscles. Lateral terminal accessory spines do not have musculature and thus any observed movement of those spines is passive. Interestingly, penile spines are connected to long, thin muscle fibers that trifurcate distally, with individual fibers attaching to each of three spines on left and right sides of segment 11. Due to size and location, it is not yet clear whether each penile spine is capable of independent movements. Although the function of a penile spine has been questioned [1, 13, 32] detection of penile-spine-specific muscle fibers indicates a potential for distinct movements of each spine during the process of mating.

The pharynx is composed of a pharyngeal bulb, encircled by multiple fibers with different but integrated functions. It is the most complex muscle system in *Echinoderes*. Radial and circular muscle fibers of the pharynx bulb are reported in many studies ([1, 41, 30] and references therein). Contraction of radial fibers increases the bulb's lumen diameter, while contraction of circular fibers decreases the lumen diameter. Together, antagonistic muscle coordination enables the pharynx to function as muscular sucking pump [1, 17, 22, 55]. The bulb's anterior sphincter is likely to have a selective function as food moves into the buccal cavity. The posterior sphincter may regulate passage of mechanically digested food into the midgut, and prevent backflow from the midgut during changes in

body pressure [32]. Pharynx retractors move the bulb in a posterior direction when the head is retracted into the body. Outer pharyngeal protractors (pop) 'pull' the pharynx bulb out of the trunk when the introvert is everted. The inner protractors (pip) also move the pharynx in an anterior direction, further toward the mouth cone. The process of protraction may be very fast, due to a combination of pharyngeal musculature (pop + pip) and the increase of internal body pressure from dorsoventral muscles (dvm) of the trunk. The pharynx suspensor muscles (phs) could act to maintain the position of the pharynx bulb, and prevent twisting, when the pharynx is retracted within the trunk. Additional musculature surrounding the pharynx bulb may assist in the movement of gut contents, such as antagonistic contraction of longitudinal and radial fibers as suggested by Neuhaus [30]. The orthogonal grid of longitudinal and circular muscles surrounding the midgut implies that digestion may be enhanced, in part, by peristalsis. This would enable particles to be displaced toward the hindgut where transverse dilatator muscles regulate defecation [32]. A circular muscle acting as an antagonist to these posterior dilatators was described in *Centroderes* sp. and *Zelinkaderes floridensis* [30]. We did not identify a hindgut circular muscle in *Echinoderes.*

Comparative myoanatomy between kinorhynchs and closely related groups

Based on morphological characters, Kinorhyncha, Priapulida and Loricifera are grouped together as the Scalidophora [10, 11, 30]. Molecular analyses have suggested an alternative hypothesis, where kinorhynchs and priapulids form a monophyletic group, excluding Loricifera [54, 12]. We predict, however, that loriciferans will be repositioned within a scalidophoran clade following new tests with improved molecular and taxonomic sampling. Thus far the most consistent interpretation is that Scalidophora (kinorhynchs + priapulids ± loriciferans) is recognized as the most basal branch within Ecdysozoa [6, 14, 16, 56, 57]. Accordingly, comprehensive descriptions of myoanatomy in *Echinoderes* and other kinorhynch genera will broaden our understanding of how different types and patterns of musculature evolved within Scalidophora, and by extension, Ecdysozoa. Yet our results also imply that new mysteries have surfaced regarding putative ancestral patterns of musculature in the lineage of animals preceding the scalidophorans. For instance, there are undeniable contrasts in the size, symmetry and complexity of scalidophoran body plans that are reflected in their respective myoanatomy. Most notable is the obvious pattern of segmentation in Kinorhyncha that is apparently absent in Priapulida and Loricifera.

Comparatively, kinorhynchs, priapulids and loriciferans have an eversible head that facilitates locomotion, feeding, and protective behavior. In kinorhynchs, the radially symmetric mouth cone and introvert are equipped with unique arrays of oral styles and rings of scalids that likely overlay an original bilateral symmetry, as observed within the trunk of all kinorhynch species [35]. The development of such radial symmetry at the anterior end is most likely an adaptation to their burrowing mode of life, which involves uniform contact with sediments [35]. Although members each of these phyla 'burrow' in sediments, the segmented trunk of kinorhynchs also differs from the body plans of priapulids and loriciferans, which are essentially vermiform and ovular, respectively. Thus, different numbers and arrangements of trunk muscles are not directly comparable among these three animal groups. Nevertheless, Kristensen and Higgins [1] suggested that, although distinctly segmented, longitudinal muscles in the trunk of kinorhynchs might be homologous to a layer of longitudinal muscles in the body of priapulids. In our opinion, direct comparisons of myoanatomy among Kinorhyncha, Loricifera and Priapulida are only possible within their head and neck regions, at least as far as our methods have revealed.

In loriciferans, the distal part of the mouth cone is arranged in a hexaradial pattern, while it is pentaradial in priapulids and kinorhynchs [39]. However, the internal arrangement of musculature does not reflect their respective patterns of mouth cone symmetry. In Kinorhyncha, there are 8-9 pairs of short longitudinal muscles in the mouth cone [26, 30], whereas there are eight individual muscles in Loricifera [36]. Based on their arrangement, Neves et al. [40] consider these muscles to be homologous in both phyla. Comparable muscle groups have not been found in priapulids. The radial arrangement of introvert scalids is roughly pentagonal in priapulids and kinorhynchs. Priapulids show twenty-five longitudinal rows of scalids in the introvert [37], whereas the kinorhynch introvert typically bears individual rings of 10-20 scalids each. Loriciferans exhibit a highly variable arrangement of

introvert scalids among genera and species, as well as among larval and adult stages. And, introvert scalid musculature is intrinsic in loriciferans, yet completely extrinsic in kinorhynchs and priapulids, resulting in the passive movements of rows or rings of scalids in the latter two taxa [1, 39]. Furthermore, there are several longitudinal and circular muscles within the introverts of both priapulids and loriciferans; however, the arrangement is different in each phylum, with a densely packed grid-like pattern of body wall muscles in priapulids, and a net-like pattern composed of few circular muscles and notably thin longitudinal fibers in loriciferans that appear to be associated with the anteriormost rows of scalids [40]. Kinorhynchs lack a grid or net-like arrangement of muscles in their introvert, and instead show a single introvert circular muscle that is integrated with several retractors.

Introvert retractors are generally referred to in the literature as inner and outer retractors, attaching to the introvert wall on both sides of the brain in each of these three phyla [1, 32, 39, 36, 37]. This arrangement has been suggested as one of several apomorphic characters for grouping scalidophorans together [18]. However, further comparative analyses of introvert musculature indicate there are several important taxon-specific differences. The so-called inner retractors of kinorhynchs are in fact part of the pharyngeal bulb musculature, and participate in retraction of the pharynx and the mouth cone, not the introvert. And the outer retractors of *Echinoderes* are introvert retractors, and therefore inner and outer retractors are assigned to separate organs. From a recent cytochemical study of myoanatomy in the loriciferan *Nanaloricus* sp., there is some evidence that dorsal and ventral longitudinal retractors of the head might correspond to the outer retractors, while the posterior part of the mouth cone retractors could correspond to the inner retractors described for *Nanaloricus mysticus* by TEM [39, 40]. However, there is an intricate system of mouth cone and buccal tube retractors that occupy a similar position, and therefore a definitive correlation is not yet possible without co-labeling musculature and nucleic acids or neurites of the loriciferan brain, or further comparative studies at the ultrastructural level. Regarding the unusual passage of muscle fibers through tissues of the brain, we did find a similar pattern in *Echinoderes* to what has been found in loriciferans, supporting previous observations [1, 36, 39]. In priapulids, inner and outer retractors were designated as short and long, and were not given positional correlations relative to brain structure [37]. So it appears that more information is required to confirm spatial relationships between organ-specific retractors and cycloneuralian brain architecture within loriciferans and priapulids. Fibers that extend along inner and outer sides of a collar-shaped brain in different taxa, but connect with non-homologous organs, may simply represent convergent solutions to the same problem. If there is a correspondence between inner (pharynx/mouth cone) and outer (introvert) retractors confirmed by co-labeling experiments in all three phyla, it may prove to be an apomorphy of Scalidophora. Until then, designating the arrangement of 'inner and outer retractors' as a unifying character should be treated with caution.

Loriciferans and kinorhynchs also share a distinct neck region with a single, thick circular muscle [1, 40, 41]. In priapulids, only larvae exhibit a well-developed neck region, whereas in adults the introvert and trunk typically join each other directly [37]. And although there is some external similarity between a priapulid larva and adult loriciferans, there is no circular muscle associated with the neck region of larval priapulids. Furthermore, larval priapulids can retract their head and neck internally within the trunk for protection, in contrast to the non-retractable neck regions of kinorhynchs and loriciferans.

In Loricifera, the pharynx is composed of myoepithelial cells with radial fibers [17, 36, 39]. Within Cycloneuralia, a myoepithelial pharynx is only shared by loriciferans and nematodes, with putative secondary losses in priapulids and kinorhynchs, however this type of pharynx most likely evolved independently several times [17, 18, 30]. The pharynx in priapulids and kinorhynchs includes both an epithelium and a muscle layer [1, 17, 18]. Among scalidophorans there are recognized differences also in the shape and orientation of the pharyngeal lumen (Y or inverted-Y). Loriciferans and homalorhagid kinorhynchs possess a triradiate sucking pharynx, however luminal orientation is a 'Y-shape' configuration in loriciferans, and an 'inverted-Y' in homalorhagid kinorhynchs [1, 17, 18, 36, 39]. In contrast, circular pharyngeal lumens are observed in priapulids and cyclorhagid kinorhynchs [1, 17, 18, 37]. As priapulids are considered the sister group to Kinorhynchs, an unsegmented body plan with a weaker pharynx type (circular lumen) may have been

characteristic of the priapulid-kinorhynch ancestor. Yet, as the triradiate sucking pharynx is also present in Kinorhyncha and Loricifera, and may be homologous in Ecdysozoa [17], it is more reasonable to envision the circular pharyngeal lumen as a derived character state that became adaptive for various feeding strategies among cycloneuralians.

Nematomorphs typically are considered cycloneuralians and hence closely related with the scalidophorans. The adult has an extremely long and slender vermiform body plan, with a reduced digestive system and a rounded anterior end often lacking a distinct head [18, 22]. Adult nematomorph features are not readily comparable with scalidophorans, however, an endoparasitic larval form is equipped with an introvert and retractable proboscis [18, 58]. Muscle groups that operate the introvert and proboscis (mouth cone) are somewhat comparable to muscle groups in the head regions of priapulids, kinorhynchs and loriciferans, although based on one study by CLSM for the nematomorph larva of *Gordius aquaticus* [58]. Musculature within the larva's anterior include six introvert retractors, and six proboscis retractors, with none of those retractors associated with circular muscles, which appear to be absent. Some similarity between loriciferans and nematomorphs is observed regarding the position and function of proboscis retractors in the *Gordius* larva and the buccal tube retractors of adult *Nanaloricus* sp. [40]. Moreover, the oblique muscles described in the Gordius larvae can have the same function as the retractors of the introvert in kinorhynchs. Overall, the numbers and arrangements of particular muscle groups in this nematomorph larva are not shared with scalidophorans, especially when comparing introvert circular muscles that are common to priapulids, kinorhynchs and loriciferans. It appears that the muscular organ systems of Scalidophora are relatively distinct from other cycloneuralian taxa, contributing to their putative monophyly.

Conclusions

The myoanatomy of *Echinoderes* is highly conserved. The following muscle groups were identified within each of five species: (i) two mouth cone circular muscles, and nine pairs of oral styles muscles in the mouth cone; (ii) ten introvert retractors, one introvert circular muscle, and fourteen introvert circular muscle retractors in the introvert; (iii) one neck circular muscle; (iv) ventral and dorsal longitudinal muscles within segments 1-10, longitudinal continuous fibers spanning subsets of segments 1-9, diagonal muscles in segments 1-8, and dorsoventral muscles in segments 3-10, all of them in the trunk; (v) a pharynx bulb composed of ten radial and ten circular muscle fibers, a sheath of pharyngeal protractors and retractors surrounding the pharynx, an orthogonal grid of longitudinal and circular fibers surrounding the intestine, and a minimum of one pair of hindgut dilators; (vi) three pairs of terminal spine muscles, and one pair of penile spine muscles in segment 11. Between species of *Echinoderes*, minor differences were observed among introvert retractor muscles and the composition of pharyngeal sheath musculature.

Within Kinorhyncha, there are common myoanatomical traits: introvert scalids are not supplied with muscles; the pharynx bulb is surrounded by a complex array of retractors and protractors forming a conspicuous sheath. There are both segmented and unsegmented muscles within the trunk. Dorsal or lateroventral spines are not associated with musculature. All terminal spines are associated with musculature except for lateral terminal accessory spines (LTAS). Kinorhyncha, Loricifera and Priapulida have common sets of anterior retractor muscles that are most likely convergent adaptations to a burrowing life style, and should not be utilized as phylogenetic characters. Within Scalidophora, the muscular organ system is comparatively more similar between kinorhynchs and loriciferans.

This study provides the first comprehensive investigation of myoanatomy in Kinorhyncha by CLSM and three-dimensional reconstruction, with comparative descriptions of the form and function of muscles systems in the head, neck and trunk regions of *Echinoderes* (Echinoderidae, Cyclorhagida). Our results build upon previous investigations by transmission electron microscopy, and have begun to address questions about the origins of complex myoanatomy in kinorhynchs, and closely related taxa. In the future, important insights must come from genomic and/or transcriptomic sequence analysis, characterization of gene expression patterns within muscle tissues and other organ systems, and a thorough study of early development in Kinorhyncha, the embryos of which

remain elusive.

Materials and Methods

Animal collection and fixation

Adult specimens of five echinoderid species were utilized for this project: *Echinoderes horni* and *Echinoderes spinifurca* were collected from the Western Atlantic Ocean along the southeast coast of Florida, USA; *Echinoderes dujardinii*, *Echinoderes hispanicus* and *Echinoderes* sp. were collected from the Eastern Atlantic along the southern coast of Portugal (Table 3). Benthic marine sediments were sampled from the intertidal zone with a shovel, or obtained offshore by deployment and retrieval of a Higgins meiobenthic dredge or an anchor dredge on board two different research vessels (RV Sunburst, Smithsonian Marine Station at Fort Pierce, Florida; RV Pagrus, CCMAR, Faro). Sampling depths ranged from shallow intertidal to 45 meters, and sediment types varied in composition among sampling stations (Table 3). Kinorhynchs were extracted from sediments following the ‘‘bubbling and blot’’ technique of Higgins [28, 49]. Live specimens were isolated, identified, and fixed with 4% paraformaldehyde (Electron Microscopy Sciences) in filtered seawater or 0.1 M phosphate buffered saline (PBS), overnight at 4°C. Following fixation, specimens were washed with multiple exchanges of 0.1 M PBS, and stored at 4°C in a solution of PBS containing 0.05% sodium azide (NaN3) to prevent microbial contamination.

Scanning electron microscopy (SEM)

Fixed specimens were dehydrated through a graded series of ethanol dilutions, and dried within a Tousimis Samdri-790 Critical Point Dryer (Tousimis Research Corp., Rockville, MD) with CO2 as an intermediate. Dried specimens were mounted on aluminum stubs, sputter coated with a gold-palladium alloy, and imaged with either a HITACHI S4800 or a JEOL JSM 6335 field emission scanning electron microscope.

Phalloidin and Propidium Iodide staining

Fixed specimens of *E. spinifurca* (n= 33), *E. horni* (n= 25), *E. dujardinii* (n= 28), *E. hispanicus* (n= 5) and *E.* sp. (n= 14) were washed and labeled in multiple species-specific experiments. For each species, specimens were washed from PBS:NaN3 solution with 3 × 15 min exchanges of 0.1 M PBS, followed by permeabilization in PBT (0.1 M PBS + 5.0% Triton X-100) for 24 hours at 4ºC. Filamentous Actin (F-actin) fibers of musculature were labeled by incubating specimens at concentrations of a 1:40 or 1:100 dilution of Alexa Fluor® 488, 546, or 633-conjugated phalloidin (Molecular Probes) in PBT. Incubation was always performed in the dark while rocking at 4ºC in glass spot plates. Total incubation times were typically 72 hrs with fresh staining solutions added after 24 and 48 hrs. For DNA staining, fixed specimens were rinsed from PBS:NaN3 solution with 3 × 15 min exchanges 0.1 M PBS, followed by 3 × 15 min exchanges of PBT (0.1 M PBS + 0.2% Triton X-100+ 0.5% BSA). These specimens were then treated with RNase A at 1.0 mg/ml PBT for 1.0 hr at 37ºC, washed with 3 × 15 min exchanges of PBT, and incubated with propidium iodide (PI) at 5.0 µg/ml PBT in the dark while rocking for a period of 48–72 hrs at 4ºC. Subsets of specimens were also co-labeled with phalloidin and propidium iodide for 48–72 hrs at 4ºC. All labeling experiments were terminated with multiple exchanges of 0.1 M PBS immediately prior mounting.

Mounting and Clearing

Prior to mounting, two or three layered strips of clear tape were each placed in parallel on one side of glass microscope slide, to elevate the placement of a coverslip above the specimens. A spot of double-stick tape was placed between the clear strips. Stained specimens were placed onto the double-stick tape and oriented during adhesion. To clear the specimens, slides were transferred through graded series of isopropanol dilutions, terminating with final immersion and mounting in a 2:1 mixture of benzyl benzoate and benzyl alcohol. A coverslip was placed across the strips of clear tape and sealed with clear nail polish. Alternatively, specimens were slowly moved through a glycerol series (20%, 40%, 60%, 80%) to prevent contraction of the trunk region, and then mounted in glycerol (80% glycerol, 0.1 M PBS), or Fluoromount G® (Southern Biotech) antifade mounting medium.

Confocal Laser Scanning Microscopy (CLSM) and 3D reconstruction

Confocal imaging was performed with an LSM 510 (Carl Zeiss Inc.), an LSM700 mounted on an Axio

Table 3 Summary of sampling and collection data for five species of *Echinoderes*

Species	Locality	Date	Geographical coordinates	Depth	Sediment type	Collecting gear
E. dujardinii	Ramalhete Ria Formosa (Portugal)	May 3rd, 2012	37º 00.00 N 7º 58.63 W	Intertidal	Mud with *Zostera* sp.	Shovel
E. hispanicus	Off shore Albufeira (Portugal)	April 28th 2012	36º 57.83 N 8º 12.63 W	45 m	Shell gravel	Higgins meiobenthic dredge/ Van Veen grab
E. horni	4 miles station Fort Pierce (USA)	June 6th 2011 and Sept 26th 2012	27º 28.19 N 80º 12.76 W	14 m	Muddy sand	Anchor dredge
	5 miles station Fort Pierce (USA)	June 6th 2011 and Sept 26th 2012	27º 30.01 N 80º 12.69 W	15 m	Shell gravel	Anchor dredge
	6 miles station Fort Pierce (USA)	June 6th 2011 and Sept 26th 2012	21º 29.18 N 80º 10.98 W	15 m	"Amphioxus sand"	Anchor dredge
E. spinifurca	3 miles station Fort Pierce (USA)	June 27th 2011and Sept 26th 2012	27º 28.33 N 80º 13.68 W	10 m	Muddy sand	Anchor dredge
	4 miles station Fort Pierce (USA)	June 27th 2011 and Sept 26th 2012	27º 28.19 N 80º 12.76 W	14 m	Muddy sand	Anchor dredge
	5 miles station Fort Pierce (USA)	June 6th 2011 and Sept 26th 2012	27º 30.01 N 80º 12.69 W	15 m	Shell gravel	Anchor dredge
	6 miles station Fort Pierce (USA)	June 6th 2011 and Sept 26th 2012	21º 29.18 N 80º 10.98 W	15 m	"Amphioxus sand"	Anchor dredge
Echinoderes sp.	Off shore Albufeira (Potugal)	April 28th 2012	36º 57.84 N 8º 12.63 W	45 m	Shell gravel	Higgins meiobenthic dredge / Van Veen grab

Imager upright microscope (Carl Zeiss Inc.), or a Leica DM 5000 CS with SP5 laser scanning unit. Confocal z-stack projections were compiled and analyzed with Fiji, v. 1.47 (Wayne Rasband, National Institutes of Health), and edited with Adobe Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA). For 3D reconstructions, z-stacks projections were surface-rendered Imaris v. 7.5.0 (Bitplane AG, Zürich, Switzerland). Schematics and figure plates were prepared with Adobe Illustrator CS4 (Adobe Systems Incorporated, San Jose, CA). The terminology used for external morphology and position follows Pardos et al. [46], Neuhaus and Higgins [13], and Sørensen and Pardos [49]; accordingly, trunk segments are numbered 1 to 11, from anterior-to-posterior. Internal structures are named in accordance with previous studies based on CLSM (see [56, 26, 27]) and TEM (see [1, 30]). The use of Kinorhyncha in the laboratory does not raise any ethical issues and therefore Regional or Local Research Ethics Committee approvals are not required.

Competing interests

The author's declare that they have no competing interests.

Author's contributions

MH contributed in project design, field collections, CLSM imaging and analysis, designing and building the images and writing of the manuscript. MJB participated in project design, field collections, CLSM imaging and analysis, and writing of the manuscript. FP assisted in writing the manuscript, data interpretation and building images. RCN contributed with field collections, CLSM imaging and analysis, and writing the manuscript. All authors read and approved the final manuscript.

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Additional files

Additional files 1-3: Videos as MP4

Additional file 1 Myoanatomy of the head of *Echinoderes spinifurca*.

Phalloidin staining and 3D reconstruction of the anterior region showing distinct muscle groups of the mouth cone, introvert and pharyngeal sheath. This specimen rotates on all three axes, beginning and ending with apical views. The introvert and mouth cone are fully extended. The pharynx is surrounded by a tulip-shaped muscular sheath (pale yellow) and is extended to reach the mouth cone (yellow). The oral styles and scalids (grayscale) do not have muscles. White lines on each side of the head correspond to the long terminal spines of this dorsoventrally curved specimen. Musculature is color-coded for the same specimen in Figure 2A-D of the manuscript.

Additional file 2 Myoanatomy of the body of *Echinoderes spinifurca*.

Phalloidin staining and 3D reconstruction of all muscle groups within the head, neck and trunk of the body. This specimen rotates 360º on its anterior-posterior axis, with anterior to the top. The introvert is partially everted. Segmented muscle groups of the trunk (ventral, dorsal, dorsoventral, diagonal) are visible in turquoise, blue, magenta and red, respectively. Autofluorescence of the cuticle (grayscale) shows eleven segments and spines of the trunk and terminal segment. The musculature is color-coded as in Figure 8 of the manuscript.

Additional file 3 Eversion, retraction and motility of *Echinoderes*.

Video of a live specimen of *E. spinifurca* under brightfield illumination. Eversion and retraction of the introvert and associated spinoscalids of the head region facilitate forward movements of the animal. The segmented trunk is highly flexible and exhibits a broad range of dorsoventral and lateral articulation. The entire gut system (mouth cone, pharynx, intestine) moves within the body as the introvert is everted and retracted. The pair of red spots at the anterior and correspond to photoreceptors.

Chapter VII

Comparative Morphology of Serotonergic-Like Immunoreactive Elements in the Central Nervous System of Kinorhynchs (Kinorhyncha, Cyclorhagida)

Morfología comparada de los elementos serotoninérgicos
en el sistema nervioso central
de los Kinorrincos (Kinorhyncha, Cyclorhagida)

MARÍA HERRANZ, Fernando Pardos, and Michael J. Boyle
Journal of Morphology 274: 258–274 (2013)

Los distintos taxones de animales Cicloneuralios presentan una arquitectura orgánica similar, con caracteres significativos para la evolución de los metazoos, aunque todavía se dispone de escasos estudios descriptivos comparados y modernos sobre las homologías celulares y moleculares en ellos. En este trabajo se inmunomarcaron y caracterizaron los elementos del sistema nervioso serotoninérgico de los kinorrincos *Echinoderes spinifurca, Antygomonas paulae* y *Zelinkaderes brightae* estudiándose posteriormente utilizando microscopía láser confocal. Se combinaron marcadores fluorescentes de ADN y observaciones de estructuras autofluorescentes para guiar las interpretaciones de la anatomía interna y externa en cada especie. Los resultados muestran un patrón común del sistema nervioso central mostrando un cerebro circunentérico dividido en dos anillos de cuerpos neuronales, anterior y posterior, y un neuropilo central, conectado a un cordón nervioso ventral longitudinal formado por múltiples fibras. Las semejanzas y diferencias en las estructuras de los sistemas nerviosos de las distintas especies se han observado y descrito, haciendo hincapié en la estructura de anillo incompleto de la región anterior del cerebro de los kinorrincos, la relación funcional entre el cerebro y el introverto móvil, y el número y disposición de las fibras nerviosas y los cuerpos neuronales del cordón nervioso ventral. Este cordón ventral se bifurca terminando en dos cuerpos celulares ventrolaterales en *E. spinifurca* y formando un asa terminal asociada con la espina medioterminal en *A. paulae* y *Z. brightae*. Se discute el posible significado funcional y filogenético de todos estos rasgos y disposiciones.

JOURNAL OF MORPHOLOGY 274:258–274 (2013)

Comparative Morphology of Serotonergic-Like Immunoreactive Elements in the Central Nervous System of Kinorhynchs (Kinorhyncha, Cyclorhagida)

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ABSTRACT Cycloneuralian taxa exhibit similar organ system architectures, providing informative characters of metazoan evolution, yet very few modern comparative descriptions of cellular and molecular homologies within and among those taxa are available. We immunolabeled and characterized elements of the serotonergic nervous system in the kinorhynchs *Echinoderes spinifurca*, *Antygomonas paulae,* and *Zelinkaderes brightae* using confocal laser scanning microscopy. Fluorescent markers targeting DNA were combined with observations of autofluorescent structures to guide interpretations of the internal and external anatomy in each species. Results show a common pattern of the central nervous system with a circumenteric brain divided into ring-shaped anterior and posterior neuronal somata and a central neuropil connected to a multi-stringed, longitudinal ventral nerve cord. Structural similarities and differences in the nervous systems of these species were observed and described, stressing the incomplete ring nature of the anterior region of the kinorhynch brain, the functional relationship between the brain and the movable introvert, and the number and arrangement of nerve strings and somata of the ventral nerve cord. The ventral cord ends in two ventrolateral cell bodies in *E. spinifurca*, and forms a terminal loop associated with a midterminal spine in *A. paulae* and *Z. brightae*. The possible functional and phylogenetic significance of these features and arrangements are discussed. J. Morphol. 274:258–274, 2013.

KEY WORDS: Kinorhyncha; nervous system; confocal laser scanning microscopy; serotonin; Cycloneuralia

INTRODUCTION

Invertebrate nervous systems consist of complex, multifaceted sensory organs that typically represent distinct clade-specific cellular and molecular architectures (Bullock and Horridge, 1965; Schmidt-Rhaesa, 2007; Richter et al., 2010). Such distinction is exemplified by the Cycloneuralia (Ahlrichs, 1995; Nielsen, 1995), a diverse invertebrate group that traditionally includes Nematomorpha, Nematoda, Priapulida, Kinorhyncha, and Loricifera (Schmidt-Rhaesa, 1998; Schmidt-Rhaesa, 2007; Nielsen, 2012). Their name makes reference to an "apomorphic" ring-like brain configuration; however, hypotheses vary as to whether cycloneuralians are considered a monophyletic group (Nielsen, 2001; Dunn et al., 2008; but see Hejnol et al., 2009) or represent a paraphyletic assemblage of taxa (Garey, 2001; Telford et al., 2008; Budd and Telford, 2009), and therefore the monophyletic status of Cycloneuralia is still open (Edgecombe et al. 2011). Importantly, if it is possible that cycloneuralians gave rise to the arthropods (Budd and Telford, 2009), then modern comparative studies describing homologous organ systems between and within cycloneuralian taxa are both necessary, and appropriately aimed at revealing new insights into the evolution of Metazoa. Here, we describe cellular and molecular elements of the central nervous system in different kinorhynch species.

Kinorhyncha is a phylum of microscopic, segmented marine invertebrates that constitute part of the meiofauna. Due to their small size (less than 1 mm in length), compound light microscopy does not provide enough resolution for describing their organ systems in detail. Previous studies focusing on their internal anatomy have been primarily based on transmission electron microscopy (TEM) (e.g., Brown, 1989; Kristensen and Hay-Schmidt, 1989; Kristensen and Higgins, 1991;

Additional Supporting Information may be found in the online version of this article.

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Nebelsick, 1993; Neuhaus, 1994; G[a] Ordóñez et al., 2000; Neuhaus and Higgins, 2002). To date, most of the studies on kinorhynchs described external features or ultrastructure of particular regions such as the introvert and mouth cone, or sensory organs. Studies are restricted to the genera *Echinoderes, Kinorhynchus,* and *Pycnophyes*: *Echinoderes capitatus* Zelinka, 1928 (Nebelsick, 1993); *Kinorhynchus giganteus* Zelinka, 1928 *and Kinorhynchus phyllotropis,* Brown and Higgins, 1983 (Moritz and Storch, 1972a, b; Brown, 1989); and *Pycnophyes greenlandicus* Higgins and Kristensen, 1988 (Kristensen and Higgins, 1991). The structure of the nervous system in kinorhynchs has been the subject of even fewer studies or was part of a general anatomical overview typically dealing with no more than three species: *Echinoderes aquilonius* Higgins and Kristensen, 1988; *P. greenlandicus* (Kristensen and Higgins, 1991); *E. capitatus* (Nebelsick, 1993); *Pycnophyes dentatus* Reinhardt, 1881; *Pycnophyes kielensis* Zelinka, 1928; and *Zelinkaderes floridensis* Higgins, 1990 (Neuhaus, 1994). Most of these data have been reviewed in Neuhaus and Higgins (2002).

The kinorhynch nervous system is generally described as an orthogonal arrangement with several longitudinal nerve cords in the trunk that are connected by two commissures per segment (Zelinka, 1928; Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus and Higgins, 2002). The brain is ring-like surrounding the anterior part of the gut and introvert retractor muscles. It is divided transversely into three regions consisting of anterior and posterior neuronal somata separated by a central neuropil. The anterior neuronal somata are organized into a 10-lobed structure with numerous perikarya forming a ventrally opened ring; the central region is a closed ring with a well-developed neuropil containing a comparatively low number of broadly distributed neuronal somata; the posterior neuronal somata contain numerous perikarya arranged in irregular accumulations (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994; Neuhaus and Higgins, 2002).

The combined use of histochemical or immunohistochemical methods and three-dimensional imaging by confocal laser scanning microscopy (CLSM) has become an important tool for describing complex organ systems in microscopic animals. Recently, detailed investigations have been performed for several interstitial invertebrate groups: Annelida (e.g., Worsaae and Rouse, 2010), Gastrotricha (e.g., Hochberg, 2007; Hochberg and Atherton, 2011), Mystacocarida (Brenneis and Richter, 2010), Rotifera (e.g., Hochberg and Gurbuz, 2008), and Priapulida (e.g., Rothe and Schmidt-Rhaesa, 2010) among others. However, thus far only three CLSM studies on Kinorhyncha have been reported: Rothe and Schmidt-Rhaesa (2004), Schmidt-Rhaesa and Rothe (2006), and Müller and Schmidt-Rhaesa (2003), which were focused on descriptions of the muscular system.

Animals use a wide variety of neurotransmitters in chemical synapses. No single transmitter type is used throughout the entire nervous system, thus it is not possible to characterize the whole nervous system using a single type of fluorescent marker (Rothe and Schmidt-Rhaesa, 2009). However, to establish a working protocol for labeling molecular components of the nervous system in kinorhynchs, it was reasonable to begin with standard markers such as antibodies against serotonin neurotransmitter molecules (5-HT; 5-Hydroxytryptamine, monoamine neurotransmitter). Our methods resulted in new data that enabled us to describe a subset of the nervous system. Due to the body wall structure of these animals having a resistant chitinous cuticle that prevents penetration of most chemical reagents such as antibodies, the application of immunohistochemical techniques with kinorhynchs represented a difficult challenge.

For this investigation we selected, examined, and compared three cyclorhagid kinorhynchs species. We discuss our observations pertaining to several associated aspects of the central nervous system of kinorhynchs that may contribute to a broader understanding of evolution in Scalidophora and Cycloneuralia. This report is the first descriptive investigation of the nervous system in Kinorhyncha based on immunolabeling technology.

MATERIALS AND METHODS

Echinoderes spinifurca Sørensen et al. (2005) and *Antygomonas paulae* Sørensen (2007) were collected in July and August 2011 from a series of designated sampling stations located offshore of the Fort Pierce Inlet, FL. The sampling stations were at intervals of nautical miles that included, three miles station—27° 28.33′ N, 80° 13.68′ W; four miles station—27° 28.19′ N, 80° 12.76′W; five miles station—27° 30.01′ N, 80° 12.69′ W; six miles station—27° 29.18′N, 80° 10.98′W. Sampling depths ranged from 13 to 15 m among stations. *Zelinkaderes brightae* Sørensen (2007) was collected only from the first two stations. Benthic marine sediment samples were collected from each station by deployment and retrieval of a Higgins anchor dredge (rectangular steel-box mouth opening: 29 cm width; 12.5 cm height) with a canvas collection bag (60 cm length). The dredge was attached to a 0.64 cm diameter cable that is connected to a hydrographic winch aboard the RV Sunburst, owned and operated by the Smithsonian Marine Station at Fort Pierce (SMSFP). Kinorhynchs were extracted from the sediment following the "bubbling and blot" technique of Higgins (Higgins, 1988; Sørensen and Pardos, 2008). Live specimens were isolated, identified, and fixed with 4% paraformaldehyde in filtered seawater overnight at 4°C. Following fixation, specimens were washed with multiple exchanges of phosphate buffered saline (PBS) and stored at 4°C in a solution of PBS + 0.05% sodium azide (NaN_3) to prevent microbial growth and contamination.

Scanning Electron Microscopy

For scanning electron microscopy (SEM), previously fixed specimens were dehydrated through a graded series of ethanol dilutions (10–100%). Specimens in 100% ethanol were dried with CO_2 in a Tousimis Samdri-790 Critical Point Dryer (Tousimis Research Corp., Rockville, MD). The dried specimens

were mounted on aluminum stubs, sputter coated with gold-palladium and imaged with a HITACHI S4800 field emission SEM (Hitachi High-Technologies America, Pleasanton, CA); coating and SEM imaging were performed at the United States Department of Agriculture (USDA), United States Horticultural Research Laboratory in Fort Pierce, FL.

Propidium Iodide Labeling

Fixed specimens of each kinorhynch species were transferred from PBS into PBT (PBS + 2.0% Triton X-100), followed by several additional exchanges of PBT. Specimens were treated with RNase A at 1.0 mg/ml PBT for 1 h at 37°C to remove RNA molecules from cells. Animals were then treated with a fluorescent stain for nucleic acids, propidium iodide (PI), at 5.0 μg/ml PBT for a period of 48–72 h at 4°C. When utilized as a singe molecular marker, or in combination with fluorescently conjugated antibodies, PI labeling was terminated with multiple exchanges of PBS prior to imaging by CLSM.

Immunohistochemistry

E. spinifurca (n = 20) was selected as the primary model in which to characterize the immunoreactivity (IR) of antibodies to serotonin (5-HT) neurotransmitter molecules. We also performed anti-5-HT treatments with specimens of *A. paulae* (n = 5) and *Z. brightae* (n = 3). The cuticle of *E. spinifurca* was permeablilized by the removal of terminal spines or penetration of the terminal segment by micro-dissection. Dissected animals were incubated in blocking solution (PBT + 0.5% bovine serum albumin + 10% normal goat serum) at 4°C overnight. Specimens were incubated with a primary, rabbit anti-serotonin (5-HT) antibody (Sigma-Aldrich) at a concentration of 1:500 in blocking solution, at 4°C for 72 h. Primary anti-5-HT was removed with multiple exchanges of PBT. Specimens were then incubated with a secondary anti-rabbit $F(ab')_2$ fragment–Cy3 antibody (Sigma-Aldrich) at a concentration of 1:500 in blocking solution, at 4°C for 72 h. Fresh preparations of primary and secondary antibodies were exchanged daily during each incubation, respectively. Secondary antibodies were removed with multiple exchanges of PBT, followed by three 15-min exchanges of PBS prior to mounting. Incubations were performed in Pyrex spot plates, in the dark, while rocking.

CLSM

Specimens labeled with PI or Cy3 secondary antibodies were attached to glass slides on a central mount of double-stick tape. Two strips of clear tape were placed on each side of the central mount to elevate the placement of a coverslip above the specimens. The slides were transferred through graded series of isopropanol dilutions, and then immersed and mounted in a 2:1 mixture of benzyl benzoate and benzyl alcohol. A coverslip was placed across the strips of clear tape and sealed with clear nail polish. Alternatively, specimens were immersed and mounted in glycerol (60% glycerol, 1× PBS) on glass slides with a coverslip elevated above the specimens by modeling clay. All labeled specimens were analyzed and imaged using a Zeiss LSM 510 confocal laser scanning microscope with Zen 2009 software (Carl Zeiss, Thornwood, NY) at the SMSFP. Optical sections and z-stack projection micrographs were compiled from LSM files with ImageJ, version 1.45 m (Wayne Rasband, National Institutes of Health). Original SEM and CLSM micrographs were edited with Adobe Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA). Schematics and figure plates were prepared with Adobe Illustrator CS4 (Adobe Systems Incorporated, San Jose, CA).

Positional information used for describing external characters, internal anatomy, functional morphology, and the associated molecular labeling of PI and 5-HT-like IR follows the terminology and taxonomic standards for kinorhynchs as stated by Pardos et al. (1998), Neuhaus and Higgins (2002), and Sørensen and Pardos (2008); accordingly, trunk segments are numbered anterior to posterior from 1 to 11.

Nervous system terminology follows the neuroanatomical glossary of Richter et al. (2010). The authors avoid using the terms forebrain, midbrain, and hindbrain introduced by previous publications describing kinorhynch nervous systems, and instead, we use anterior neuronal somata, central neuropil, and posterior neuronal somata, respectively.

The functional anatomy of the introvert in kinorhynchs, and other cycloneuralians, may complicate the interpretation of particular structures regarding their position in the trunk and their relationship with other organs. It is important to consider that anterior organs, such as the gut and brain, move when the introvert is extended or retracted. And variation in the response of specimens to chemical fixation makes it difficult to determine the precise degree of extension or retraction of the introvert at the moment of death. Consequently, the brain and associated structures of the nervous system may be observed in different positions. When the introvert is fully withdrawn, the brain may be located towards segment 3; however, when the introvert is totally extended, the anterior margin of the brain is situated just below the first scalid row. For additional clarification in this study, we use in the term *cord* when referring to the entire bundle of midventral, longitudinal neurites with more or less defined perykarial aggregations. Individual longitudinal ventral neurites that are shown with different arrangements in our images will be referred to as *strings*.

RESULTS

E. spinifurca, *A. paulae,* and *Zelinkaderes brightae* differ in their external morphology (Fig. 1, Supporting Information Fig. S1), and internal anatomy. Microscope analyses and imaging of species-specific external and internal characters reveal several correlations with our results from molecular labeling experiments.

PI Labeling

DNA labeling with PI shows a high concentration of cell nuclei surrounding the pharynx, which marks the location of the brain in all species under investigation (Figs. 2A, 3A, 4A). These cell nuclei are arranged in three distinct groups from anterior to posterior. In *E. spinifurca*, the first group exhibits a wide bilaterally symmetric, 10-lobed structure in association with the first ring of introvert scalids (spinoscalids). This structure has a gap in its midventral region (Fig. 2C, D) and represents the position of an aggregation of anterior neuronal somata. In *A. paulae,* the 10-lobed structure is less distinct with relatively large cell nuclei, and it forms a ring in the shape of a horseshoe that is midventrally opened (Fig. 3B, C). Specimens of *Z. brightae* could not be examined in orthogonal sections. The second group of nuclei corresponds with the position of the central neuropil and presents a comparatively lower concentration of nuclei in each of the three genera studied (Figs. 2A, E, 3A, C, 4A). In each species, the third group of nuclei contains a high concentration in several irregular lobes and corresponds with the position of an aggregation of posterior neuronal somata (Figs. 2A, 3A, D, 4A).

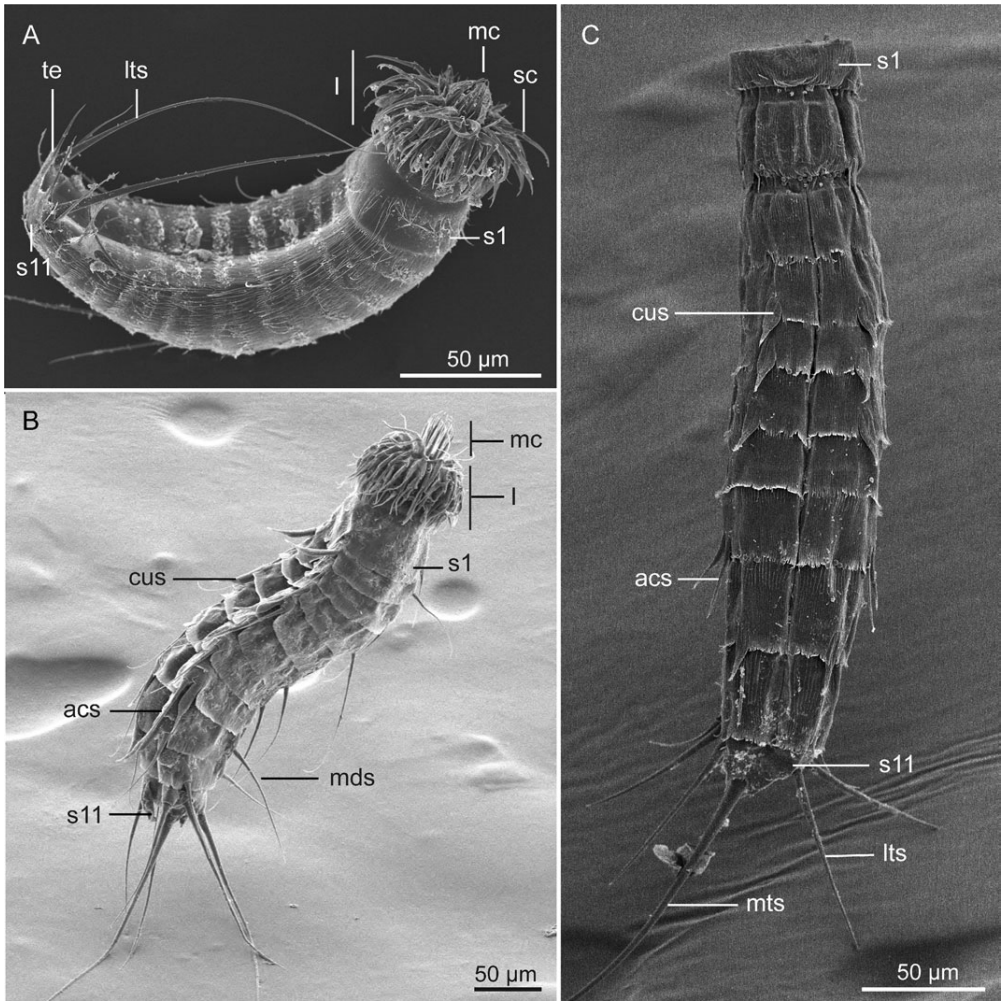

Fig. 1. Scanning electron micrographs of the external anatomy of three cyclorhagid kinorhynch species. (**A**) *E. spinifurca* in ventrolateral view with anterior to the right and introvert extended; (**B**) *A. paulae* in lateral view of with anterior to the top right and introvert extended; (**C**) *Z. brightae* in ventral view with anterior to the top and introvert retracted. acs, acicular spine; cus, cuspidate spine; I, introvert; lts, laterotermínal spine; mc, mouth cone; mds, middorsal spine; mts, midterminal spine; sc, scalid; s1, segment one; s11, segment eleven; and te, tergal extension.

There is a concentration of cell nuclei arranged longitudinally from anterior to posterior along the midventral line in the trunk segments of *E. spinifurca*, *A. paulae,* and *Z. brightae*, which likely represent the ventral nerve cord (vnc). In *E. spinifurca*, longitudinal strands of nuclei diverge from the midline in segment nine, and exhibit an inverted *Y*-shaped pattern that terminates at ventrolateral positions in segment 10 (Fig. 4A). In contrast, *A. paulae* and *Z. brightae* show a loop of cell nuclei extending ventrally from segment 10 to segment 11 (not shown).

Anti-Serotonin (5-HT) Antibody Labeling

Positive anti-serotonin-like IR was detected for several elements of the central nervous system in *E. spinifurca* (Figs. 4–7), *A. paulae,* and *Z. brightae* (Figs. 8, 9). Permeabilization of the cuticle in *Z. brightae* was problematic for antibody penetration and therefore labeling was incomplete. Autofluorescence of the kinorhynch cuticle (Supporting Information Fig. S1) as well as nonspecific binding of secondary antibodies to the scalids and some portions of the epidermis also made overall signal detection difficult.

Fifteen out of 20 specimens of *E. spinifurca* showed positive staining with a common pattern of anti-5-HT-like IR characterized by several anterior nerve rings and a ventral longitudinal nerve cord (Figs. 4B, 5, 6). When the introvert is retracted, there are two serotonergic-positive rings that are each incomplete on their respective ventral sides, and they are situated between trunk segments 2 and 3; when the introvert is extended, these rings are positioned at the level of the second row of scalids (Figs. 6B–D, 7A). The first incomplete ring

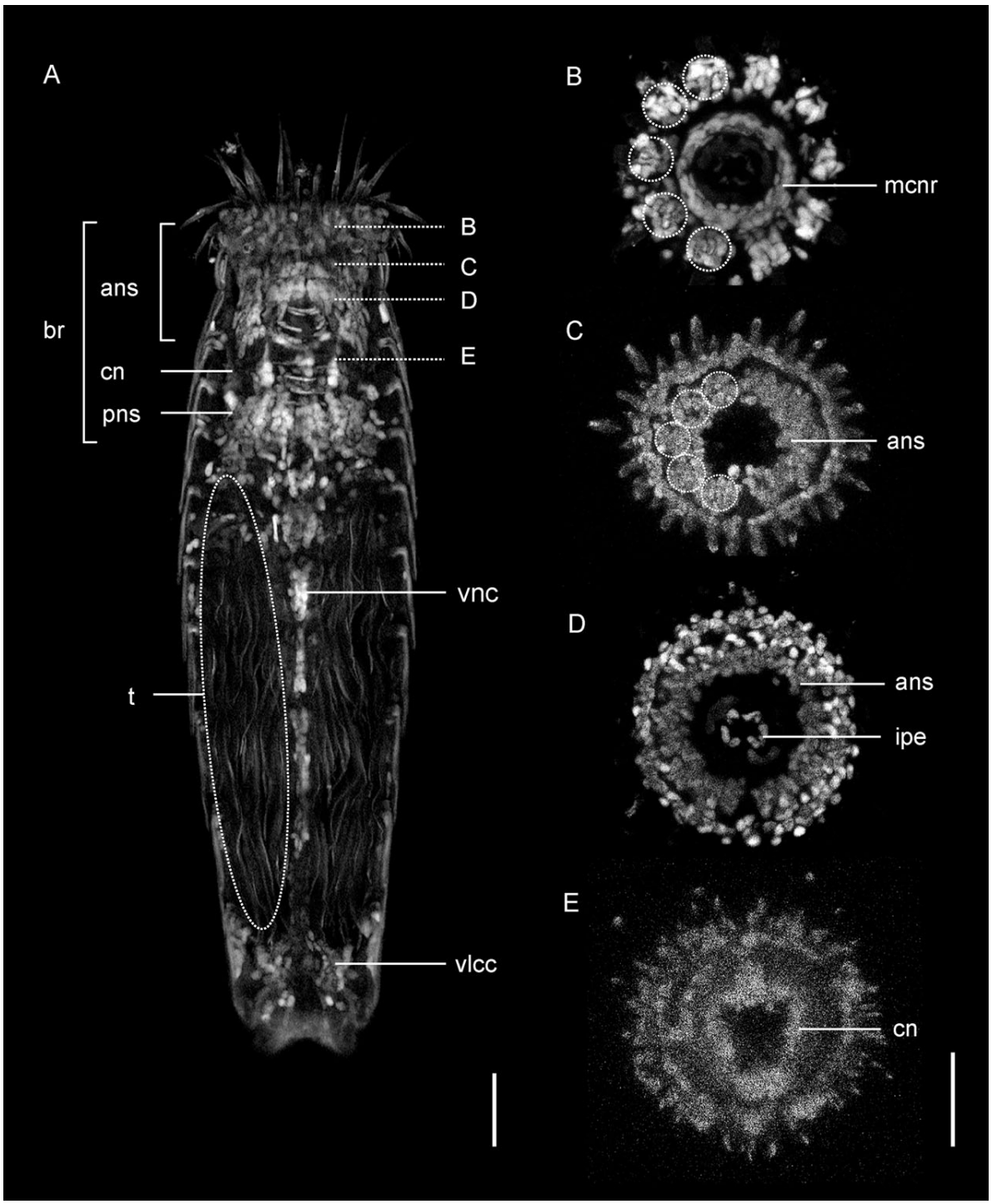

Fig. 2. Propidium iodide labeling of DNA in *E. spinifurca*. The images are confocal z-stack projections. (**A**) ventral view of a male specimen with anterior to the top; (**B–E)** optical cross sections through different brain regions corresponding to the dotted lines indicated with B, C, D, and E in image A; each cross section is orientated with ventral side to the bottom. Dashed circles in B and C indicate distinct clusters of cell nuclei in the anterior neural somata. Visible scalids and segmented cuticle outlines are autofluorescent. Testes (t) are identified as long internal sacs filled with elongate, wavy nuclei within segments 4–10 flanking the gut. Scale bars: 20 μm. ans, anterior neuronal somata; br, brain; cn, central neuropil; ipe, internal pharynx epithelium; mcnr, mouth cone nerve ring; pns, posterior neuronal somata; t, testes; vlcc, ventrolateral cell cluster; and vnc ventral nerve cord.

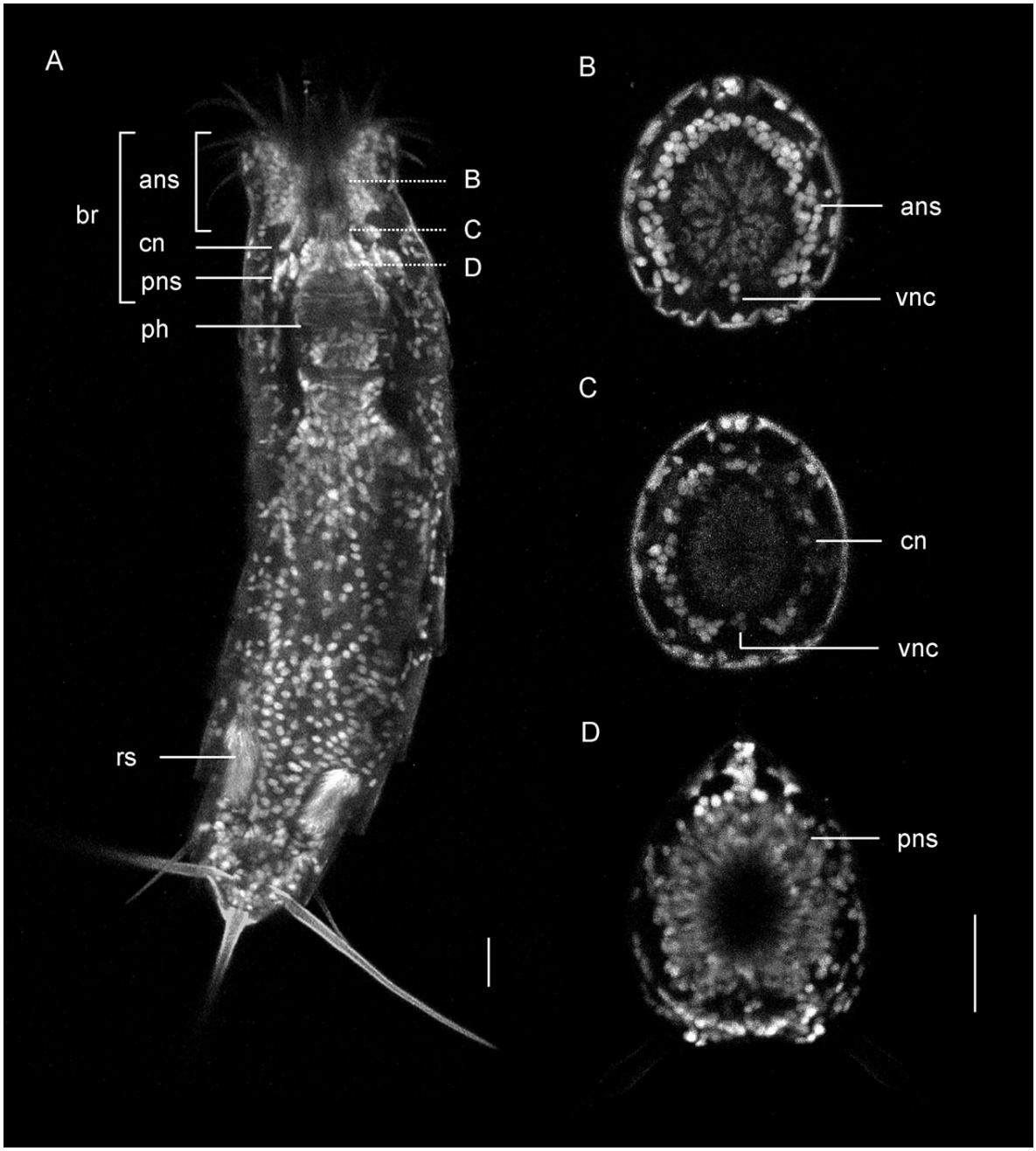

Fig. 3. Propidium iodide labeling of DNA in *A. paulae.* The images are confocal z-stack projections. (**A**) ventral view of an adult female with anterior to the top; the brain is regionally divided into two neural somata aggregations separated by a central neuropil. Female seminal receptacles appear as posterior sacs filled with elongate nuclei; (**B–D)** optical cross sections through different brain regions corresponding to the dotted lines indicated with B, C, and D in image A; each cross section is orientated with ventral side to the bottom. Visible scalids, spines, and segmented cuticle outlines are autofluorescent. Scale bars: 20 μm. ans, anterior neuronal somata; br, brain; cn, central neuropil; ph, pharynx; pns, posterior neuronal somata; rs, receptaculum seminis; and vnc, ventral nerve cord.

(inr_1) is shaped like a bracelet or necklace, which is ventrally opened and extends from two relatively large, distinct ventromedial somata (vms) showing high levels of anti-5HT-like IR (Figs. 4B, 6, 7); possible additional somata could be associated with this ring. The second incomplete ring (inr_2) lacks distinct ventromedial somata and is adjacent to the first ring, and together they form a structural ring complex (rc) putatively assigned to the central neuropil, although large, conspicuous ventromedial

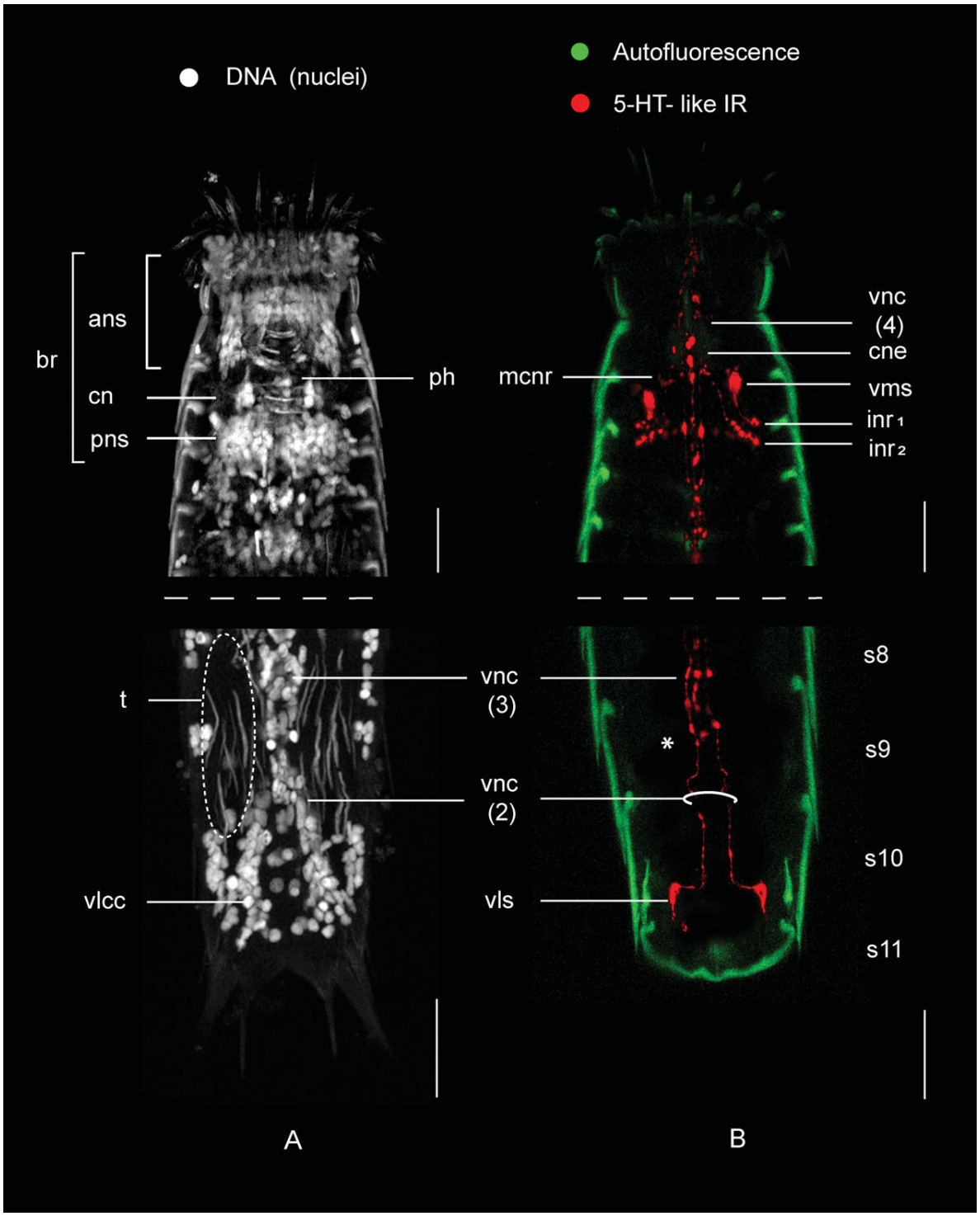

Fig. 4. Comparison of propidium iodide labeling and serotonin-like immunoreactivity in the central nervous system of *E. spinifurca*. Each specimen is oriented in ventral view with anterior to the top. (**A**) confocal z-stack projection of cell nuclei in the brain and ventral nerve cord; (**B**) confocal z-stack projection of serotonergic-like elements in the brain and ventral nerve cord. Dashed horizontal lines separate the five anterior segments from the four posteriormost segments (s8–s11) in each specimen; central segment regions are not shown. Variation in the position of brain structures between specimens A and B is the result of independent retraction of introvert and mouth cone musculature. The positions of posterior ventral nerve cord structures in A and B are similar. Visible scalids and body segment divisions are autofluorescent. Scale bars: 20 μm. ans, anterior neuronal somata; br, brain; cn, central neuropil; cne, convergent neurite; inr_1, first incomplete ring; inr_2, second incomplete ring; mcnr, mouth cone nerve ring; ph, pharynx; pns, posterior neuronal somata; s, segment; t, testes; vlcc, ventrolateral cell cluster; vls, ventrolateral somata; vms, ventromedial somata; vnc, ventral nerve cord; numbers in parentheses indicate the number of detectable vnc neurites at those locations, an asterisk marks the posterior end of the central neurite in the vnc.

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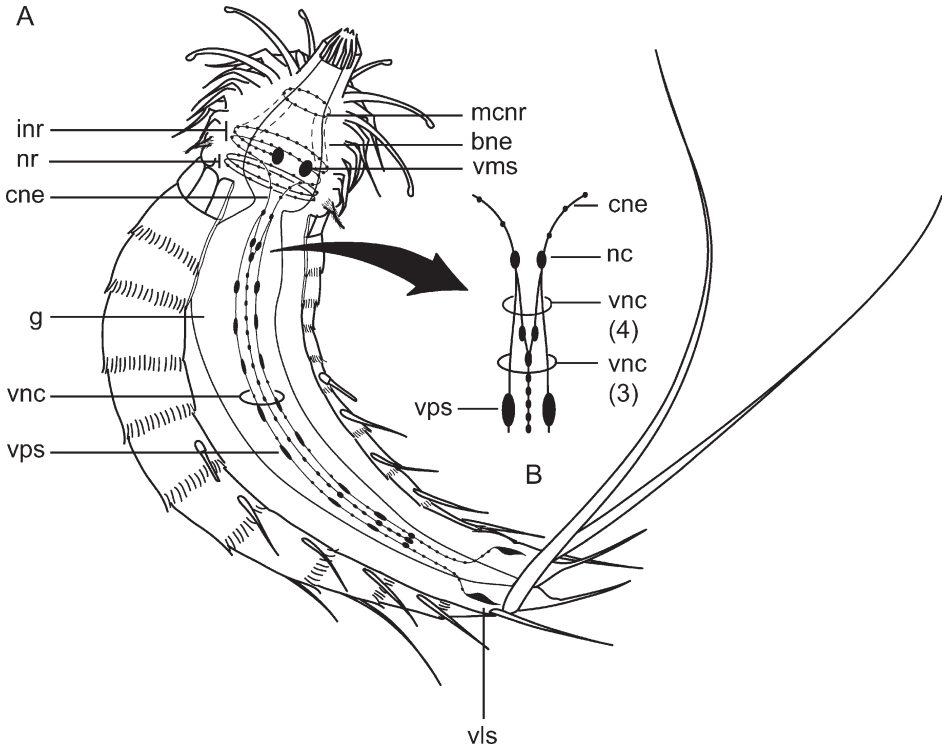

Fig. 5. Schematic drawing of serotonin-like immunoreactive elements in the central nervous system of *E. spinifurca*. (**A**) ventrolateral view of an adult with anterior to the top; both the introvert and mouth cone are extended; (**B**) enlarged view of the anterior ventral nerve cord showing the transition of four neurite strings into three neurite strings. bne, basket neurite; cne, convergent neurites; g, gut; inr, incomplete rings; mcnr, mouth cone nerve ring; nc, nerve cluster; nr, nerve rings; vls, ventrolateral somata; vms, ventromedial somata; vnc, ventral nerve cord; and vps, ventral paired somata.

somata appear to lie within the anterior neuronal somata. 5-HT-like labeling distinguishes these rings as individual units. The two rings are connected to a smaller ring, a mouth cone nerve ring (mcnr), which is fixed at the base of the mouth cone through fine neurites, and although weakly labeled, together they form a basket-like configuration characterized by these fine basket neurites (bne) and the nerve ring (Figs. 5, 6A, 7). In *E. spinifurca*, the basket-like structure is movable and always observed anterior to the two incomplete rings described above (Figs.4B, 5A, 6A, E, 7). However, when the introvert is retracted in *A. paulae* and *Z. brightae*, the smaller ring (mcnr) of the basket-like structure is positioned posteriorly in the trunk at the fifth segment level (Figs. 8A, 9A, 10A, B). The general appearance of the incomplete rings resembles a "string of pearls" that putatively contains a distribution of 5-HT-like IR synaptic vesicles along the ring-shaped neurites. An additional, complete double ring ($nr_{3,4}$) is situated posterior to, and parallel with, the incomplete rings mentioned above (Figs. 5A, 6B, E, 7, 10C). This double ring is weakly labeled and varies among individuals, but can be putatively assigned to the central neuropil. The entire ring complex (rc) (brain, mouth, and mouth cone nerve ring) may be altered in its location depending upon the position of the introvert. This is not the case for the ventral nerve cord, which is fixed to the trunk body wall (Figs. 6B–D, 7).

The second incomplete nerve ring (inr_2) is not continuous on its ventral side, where it joins two convergent neurites (cne) midventrally at the transition between the brain and ventral nerve cord (Figs. 4B, 5, 7). At this transition, each side of the ring extend parallel into a nerve cluster (nc) that joins with neurites of the ventral nerve cord on its posterior end. Movement of the ring complex during extension and retraction of the introvert determines the anterior–posterior position of the convergent neurites (Figs. 6B–D, 7). From this point, each of the two neurites branch to form a total of four strings for a short distance (Fig. 5B). The two inner strings join to form a single central neurite that is flanked by two outer strings (Fig. 5B). This is the transition where the strings become attached to the body wall and maintain a fixed position independent of extension or retraction of the introvert (Figs. 5B, 6B–D).

This arrangement constitutes the ventral nerve cord extending along the trunk toward the posterior end of the animal (Figs. 5–7). It should also be

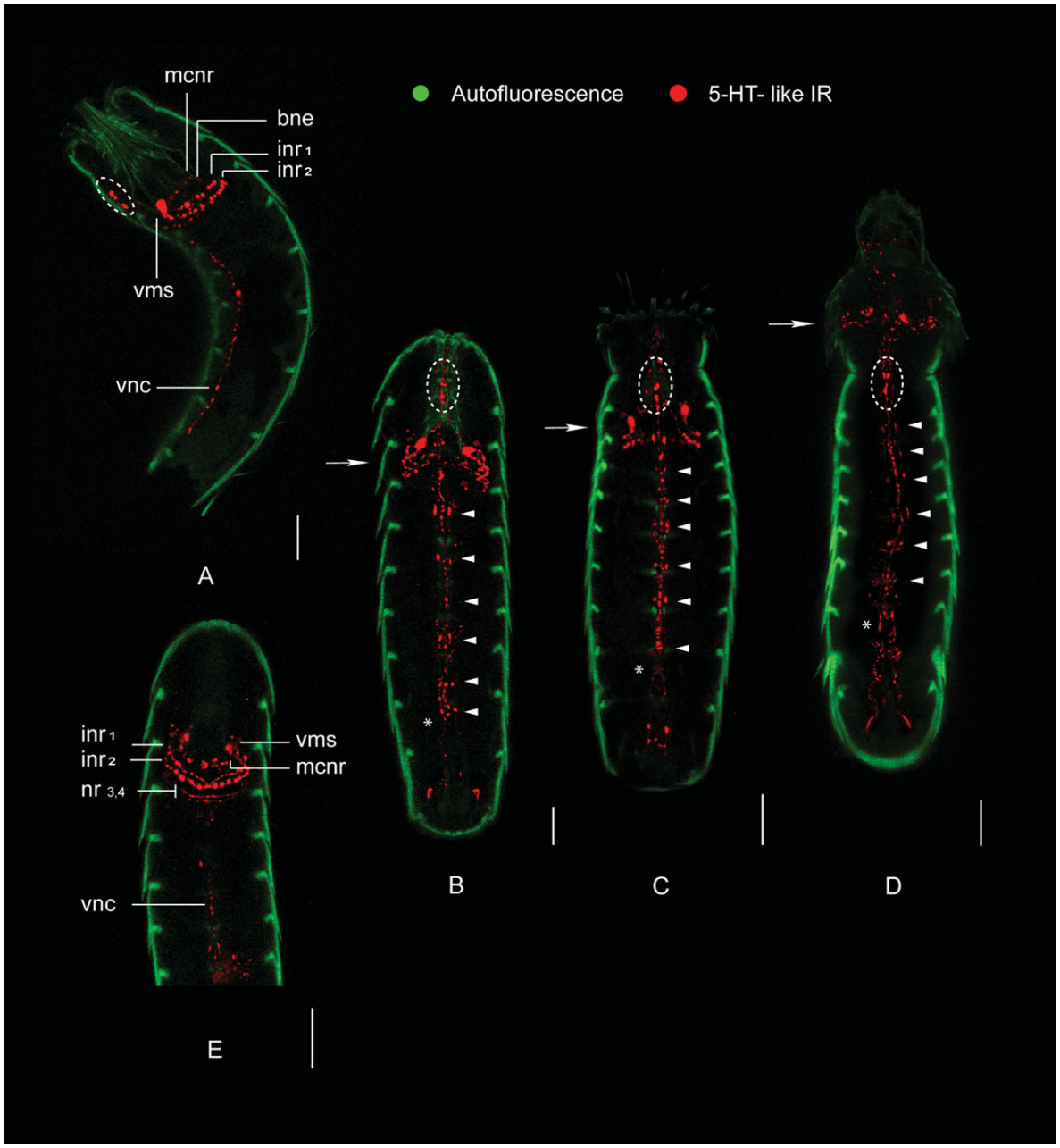

Fig. 6. Positional comparisons of serotonin-like immunoreactive elements in the central nervous system of *E. spinifurca*. The images are confocal z-stack projections. (**A**) lateral view with anterior to the top; when the introvert is retracted, the mouth cone nerve ring (mcnr) is anterior to the incomplete nerve rings ($inr_{1, 2}$); (**B–D**) ventral views showing the position of anterior 5-HT-like IR elements relative to changes in the amount of introvert extension; anterior is to the top. The position of the ring complex (arrows) relocates during extension and retraction of the introvert; (**E**) dorsal view showing relative positions of several anterior 5-HT-like IR elements. The anterior end of the ventral nerve cord is attached to the body within the first segment (dashed circles) and does not relocate during extension and retraction of the introvert. Distinct groups of neuronal cell bodies (arrowheads) are present in each segment along the vnc. Visible scalids and body segment divisions are autofluorescent. Scale bars: 20 μm. an asterisk marks the posterior end of the central neurite in the vnc. bne, basket neurite; $inr_{1,}$ first incomplete ring; $inr_{2,}$ second incomplete ring; mcnr, mouth cone nerve ring; vms, ventromedial somata; and vnc, ventral nerve cord.

noted here that ventral paired neuronal somata (vps) are segmentally arranged along the ventral cord in segments 1–9 and they are most distinct on the two lateral strings. The central string shows many regularly spaced somata, but they are not grouped in a segmental pattern (Figs. 5, 6B–D). Within segment 9, the midventral string terminates in a single cell body, while the two lateral

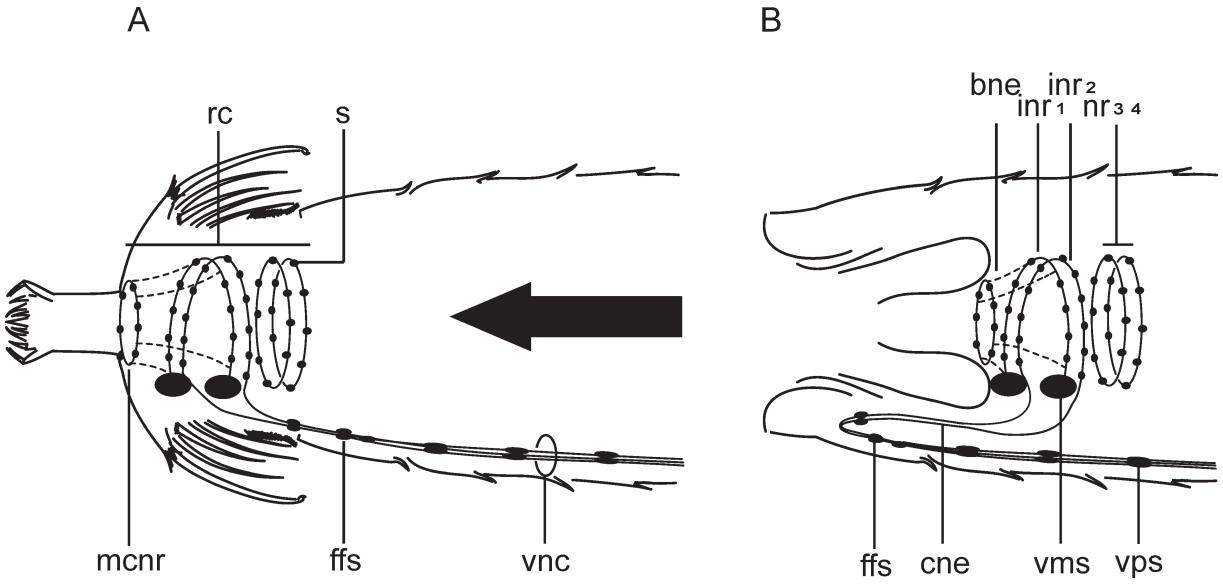

Fig. 7. Schematic drawing of serotonin-like immunoreactive elements in the anterior central nervous system of *E. spinifurca*. (**A**) lateral view with ventral side down and anterior to the left; introvert is extended. (**B**) lateral view with ventral side down and anterior to the left; introvert is retracted. The ring complex (rc) and convergent neurites (cne) relocate in association with introvert movements, whereas the ventral nerve cord remains attached at the first fixed somata (ffs). The central neuropil is not shown relative to the position of anterior incomplete rings and posterior rings. Dashed lines indicate the location of basket neurites, which typically exhibit lower levels of immunoreactivity. bne, basket neurite; ffs, first fixed somata of the ventral nerve cord; cne, convergent neurite; inr_1, first incomplete ring; inr_2, second incomplete ring; mcnr, mouth cone nerve ring; $nr_{3,4}$, nerve rings 3 and 4; rc, ring complex; s, soma; vms, ventromedial somata; vnc, ventral nerve cord; and vps, ventral paired somata.

strings continue posteriorly to segment 10, where they diverge and terminate in two ventrolateral somata (vls) that are relatively large and distinct (Figs. 4B, 5A, 6B–D). Anti-5-HT-like IR of transverse connections or commissures was not clearly detected between elements of the three ventral strings. We also did not detect neurites or anti-5-HT-like labeling of structures emerging from either caudal aggregations or somata of the ventral nerve cord. Similarly, no other longitudinal nerve strings or bundles were found. Nevertheless, positive anti-5HT-like IR exhibits intensity variation among specimens, so we cannot exclude the possibility that other rings or bundles were overlooked or weakly stained.

Specimens of *A. paulae* and *Z. brightae* also show positive anti-5-HT-like IR with a similar overall pattern to *E. spinifurca* (Figs. 8A, 9A), although differences between the three species can be detected. *A. paulae* exhibits at least two additional pairs of midlateral somata (mls) connected to the incomplete anterior nerve rings through very fine neurites (Figs. 8A–B, 10B). Only one pair of such midlateral structures can be detected in *Z. brightae* (Figs. 9A, 10A). The ventral nerve cords in both *Z. brightae* and *A. paulae* are composed of four separate neurites containing abundant somata along the entire trunk length, although a paired arrangement of neuronal somata reflecting the morphology of segmental ganglia is not apparent (Figs. 8A–C, 9A, B). The two central neurites terminate in a single neural soma within segment 9, whereas lateral neurites extend posteriorly to two slightly divergent somata in segment 10. From these somata, a conspicuous circular neurite or terminal neural loop (tl) occupies the posteriormost segment in specimens of those two species (Figs. 8C, 9A, B).

DISCUSSION

Brain

As reported by Kristensen and Higgins (1991) using TEM, the brain is divided from anterior to posterior into three ring-like regions. The anterior and posterior regions contain multiple neuronal cell bodies (perikarya), whereas the central neuropil contains fewer perikarya and more fibers when compared with the other two brain regions (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994; Neuhaus and Higgins, 2002). Using DNA labeling, we found that patterns of cell nuclei in *E. spinifurca*, *A. paulae,* and *Z. brightae* confirm the descriptions mentioned above. It was possible to identify a high concentration of neuronal cell bodies in the anterior part of the trunk surrounding the pharynx and to distinguish three ring-like aggregations of perikarya, showing that the anterior and posterior regions clearly contain more perikarya than the central neuropil. The 10-

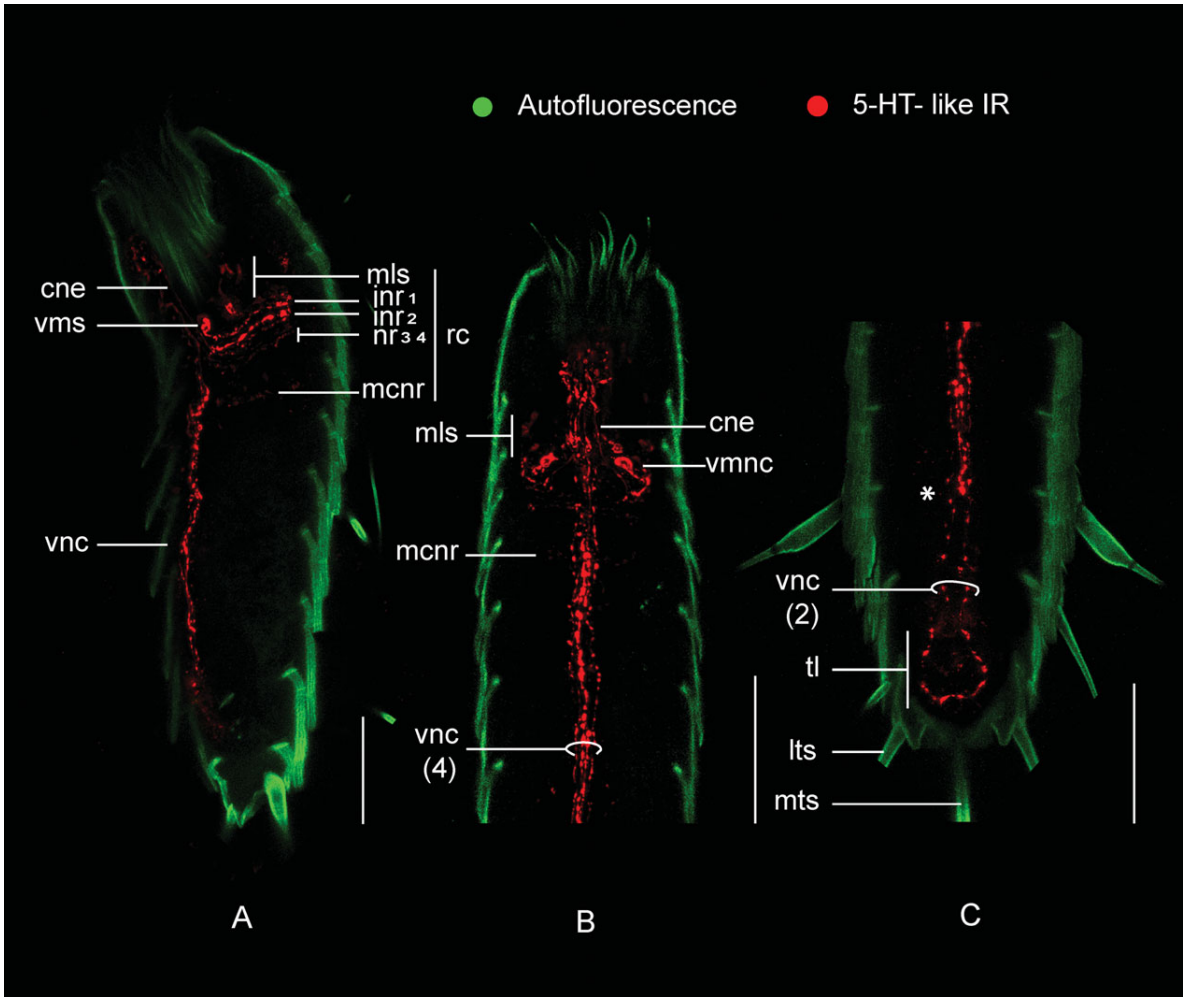

Fig. 8. Serotonin-like immunoreactivity in the central nervous system of *A. paulae*. The images are confocal z-stack projections. (**A**) lateral view with anterior to the top; introvert is retracted; (**B**) ventral view of segments 1–8 with anterior to the top; introvert is retracted; (**C**) ventral view of posterior segments 7–11; the terminal loop of the vnc extends between segments 10–11. An asterisk marks the posterior end of the central neurite in the vnc. Numbers in parentheses indicate the number of detectable vnc neurites at those locations. Visible scalids, spines and body segment divisions are autofluorescent. Scale bars: 50 μm. cne, convergent neurite; inr_1, first incomplete ring; inr_2, second incomplete ring; lts, laterоterminal spine; mcnr, mouth cone nerve ring; mls, midlateral somata; mts, midterminal spine; $nr_{3,4}$, nerve rings 3 and 4; rc, ring complex; tl, terminal loop; vms, ventromedial somata; vnc, ventral nerve cord.

lobed division reported by Kristensen and Higgins (1991) for *E. aquilonius* is identifiable in *E. spinifurca*, most notably in the anterior neuronal somata. This division was not as distinct in *A. paulae*, and no transverse sections were characterized from specimens of *Z. brightae*. All species under investigation indicate the circumpharyngeal brain is not a completely closed ring, and reveal the appearance of a horseshoe-shaped pattern in the aggregations of anterior neuronal somata. This was previously reported for *E. capitatus* (Nebelsick, 1993).

The serotonin-like positive labeling shows a subset of the nervous system. The presence of four rings, two of them midventrally incomplete, located between segments two and three (with the introvert withdrawn) was consistent in all specimens of *E. spinifurca* with detectable labels. Very similar results were obtained with specimens of *A. paulae* and *Z. brightae*. Because it is hypothesized that serotonin is the primary myoexcitatory neurotransmitter in several invertebrate groups (Hochberg, 2009), it is reasonable to assume that the ring complex of kinorhynchs may be involved in locomotory processes. However, serotonin may also be activating 5-HT receptors on the membranes of different cell types within or outside of the central nervous system. Additional labeling in other potential cell types or associated organ systems containing 5-HT-like targets was not detected, or

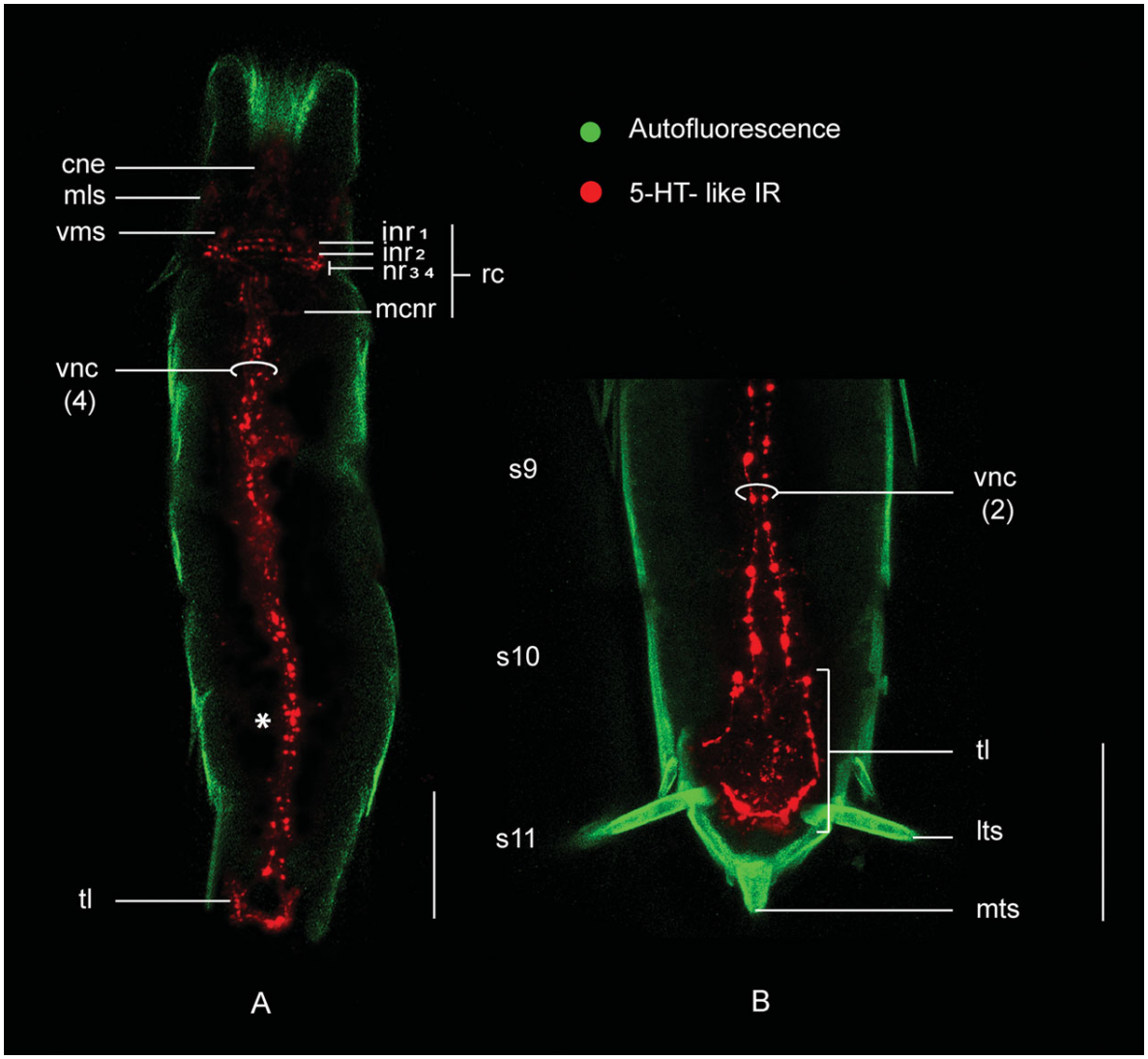

Fig. 9. Serotonin-like immunoreactivity in the central nervous system of *Z. brightae*. The images are confocal z-stack projections. (**A**) ventral view with anterior to the top; the introvert is retracted and the posterior region of segment 11 has been removed; (**B**) ventral view of the posterior segments (s9, s10, s11) with anterior to the top; the terminal loop of the vnc extends between segments 10 and 11, and appears to be damaged on the right side. An asterisk marks the posterior end of the central neurite in the vnc. Numbers in parentheses indicate the number of detectable vnc neurites at those locations. Visible scalids, spines and body segment divisions are autofluorescent. Scale bars: 50 μm. cne, convergent neurite; inr_1, first incomplete ring; inr_2, second incomplete ring; lts, lateral terminal spine; mcnr, mouth cone nerve ring; mls, midlateral somata; mts, midterminal spine; $nr_{3,4}$, nerve rings 3 and 4; rc, ring complex; tl, terminal loop; vms, ventromedial somata; vnc, ventral nerve cord.

may not be detectable, with the combination of primary anti-5-HT and secondary antibodies applied during this investigation.

Although kinorhynchs are known to have several neurites innervating the introvert scalids (Kristensen and Higgins, 1991; Nebelsick, 1993), we were not able to label any of them. A mechanoreceptor and chemoreceptor sensory function for introvert scalids has been advanced by Moritz and Storch (1972a, b) and Kristensen and Higgins (1991). The latter authors also suggest a locomotory function. However, no muscles appear to be directly associated with introvert scalids (Kristensen and Higgins, 1991; Müller and Schmidt-Rhaesa, 2003), and our results with serotonin-like labeling do not support a role in the direct motor control of scalids from the nervous system. Because it is clear that the introvert scalids contribute to forward movement of the animal, we conclude that the motion of scalids may be indirect, through contraction of dorsoventral muscles in the trunk that increase internal body pressure (Zelinka, 1928). We further hypothesize that stimulation for the muscular contraction of introvert

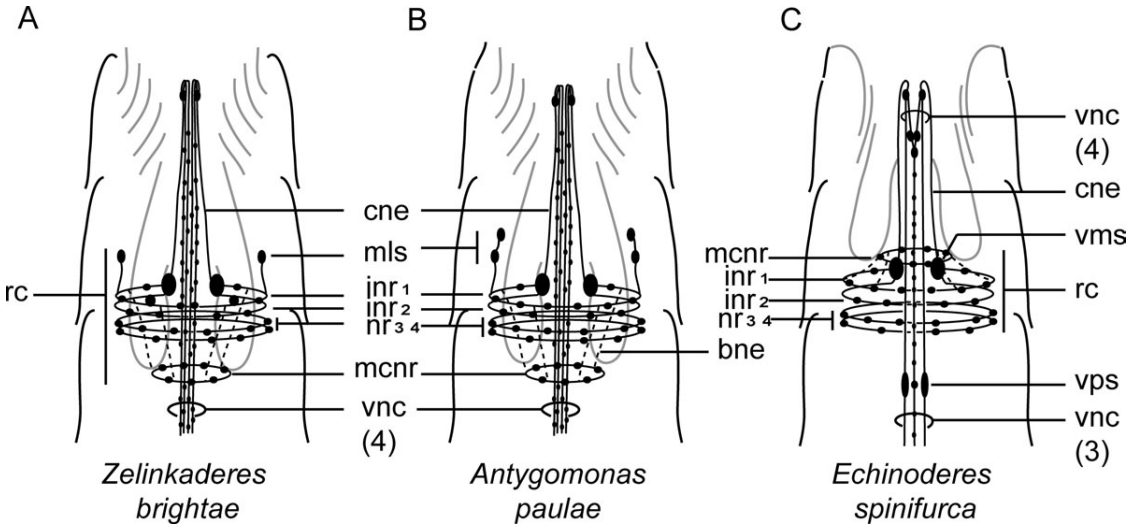

Fig. 10. Schematic drawing of comparative serotonin-like immunoreactivity in the anterior central nervous system of three cyclorhagid kinorhynch species. (**A**) *Z. brightae*; (**B**) *A. paulae*; (**C**) *E. spinifurca*. Ventral views with anterior to the top. Compared with *E. spinifurca*, the depth of introvert and mouth cone retraction within *Z. brightae* and *A. paulae* is greater (gray lines), which relocates the mouth cone nerve ring (mcnr) to the posterior end of the ring complex. The number of neurites in the ventral nerve cord is also different between *E. spinifurca* and the other two species. Dashed lines represent the presence of the basket neurites. bne, basket neurite; cne, convergent neurite; inr_1, first incomplete ring; inr_2, second incomplete ring; mcnr, mouth cone nerve ring; mls, midlateral somata; $nr_{3,\ 4}$, nerve rings 3 and 4; rc, ring complex; vms, ventromedial somata; vnc, ventral nerve cord; vpg, ventral paired somata.

retractors is accomplished by the ring complex and, especially, the ventromedial pair of neuronal cell somata.

In contrast to the introvert scalids, the nine oral styles of the mouth cone do possess muscles (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994). Accordingly, we find an anti-5HT-like immunoreactive nerve ring in the base of the mouth cone, which moves forward and backward with the protrusible mouth cone and is connected to the incomplete nerve rings by the "basket neurites" referred to above. It is remarkable that the functional differences between an eversible introvert and a protrusible mouth cone may be apparent in components of the nervous system controlling their movements.

Innervation of pharynx musculature could not be detected with anti-5-HT labeling and CLSM, although Nebelsick (1993) and Neuhaus (1994) describe the presence of nerves extending from the mouth cone ring to the pharynx. The special nature of this contractile epithelium may indicate the existence of another neurotransmitter that is different from serotonin and therefore, no pharyngeal-associated innervation was detected in this study. It is also possible that our methods for the labeling and detection of fine-scale pharyngeal innervation were not adequate.

Ventral Longitudinal Cord

High levels of anti-5-HT-like IR were shown in the ventral nerve cord and large posterior somata of all species under investigation. The presence of a ventral ganglionated nerve cord in kinorhynchs was first recognized by Claparède (1863) and also reported on by subsequent authors (e.g., Zelinka, 1928; Kristensen and Higgins, 1991; Neuhaus and Higgins, 2002). Kristensen and Higgins (1991) identified a segmentally paired midventral chain of ganglia in *E. aquilonius* and *P. greenlandicus* using TEM. Nebelsick (1993) added the presence of a caudal ganglion in her description of the nervous system of *E. capitatus*. These descriptions fit with the serotonin-like labeling of cells and neuropil characterized here in *E. spinifurca*. However, our results show four longitudinal strings in the anteriormost part of the ventral cord that transition to become three strings in segment 2 by the joining of the two central strings. These results are partially supported by those from *E. capitatus* (Nebelsick, 1993), in which the ventral cord has a double origin in the region of the introvert and becomes unpaired in the remaining trunk. These observations imply that the basic structure may be the same in both *E. spinifurca* and *E. capitatus*, and that CLSM techniques enable a finer resolution than three-dimensional reconstruction from TEM transverse sections. The ventral nerve cord in *E. capitatus* arises from the anterior neuronal somata (when the introvert is withdrawn) and bends toward the ventral side to extend posteriorly toward the caudal end (Nebelsick, 1993). In our material, the ventral nerve cord appears to originate from either the posterior side of the anterior neuronal somata, or from the central neuropil; the

exact position could not be determined precisely as double staining with PI and anti-5-HT antibodies was not performed. However, Kristensen and Higgins (1991) point to the posterior neuronal somata as the origin for the ventral cord. Our detection of two anterior, incomplete neuronal rings, that connect with the ventral nerve cord, and which may be associated with anterior neuronal somata, is more in line with Nebelsick's observations.

No additional nerve cords along the body were labeled; however, this does not necessarily mean that such structures are absent. Our results do not reflect the orthogonal nervous system described previously with TEM showing eight additional longitudinal nerve cords connected with circular nerve fibers in each segment (Kristensen and Higgins, 1991 in *E. aquilonius*; Nebelsick 1993 in *E. capitatus*). Twelve longitudinal nerve cords have been reported in *Z. floridensis* (Neuhaus, 1994); only seven or eight longitudinal cords have been reported in the genus *Pycnophyes* (Kristensen and Higgins, 1991; Neuhaus, 1994). No information on the subject is known from the genus *Antygomonas*. It should also be noted that the available published information does not address the double, single, or fused nature of the ventral nerve cords described in this study, with the exception of Nebelsick (1993) in *E. capitatus* and illustrated by Neuhaus (1994) in *Z. floridensis* and *P. dentatus*.

It cannot be excluded that a lack of signal in dorsal and lateral regions of the trunk segments is due to low levels of endogenous serotonin molecules or anti-5-HT antibodies. However, as stated above, serotonin may only appear in regions associated with muscular control implying that the remaining cords may have other functions, which could be primarily sensory. A midventral nerve cord has been previously described through TEM as innervating the segmental muscles and did not appear to have sensory function (Kristensen and Higgins, 1991), a statement in strong agreement with our results. Neuhaus (1994) and Neuhaus and Higgins (2002) described the neuromuscular junction through cell processes from muscle cells to elements of the nervous system. Our results from CLSM confirm this fact, because no neurites can be seen extending laterally from the ventral cord to the dorsoventral muscles. Further research that incorporates labeling for additional sensory neurotransmitters would be needed to confirm the functional role of the remaining longitudinal cords and would also serve to clarify their structural relationships with different sensory organs (sensory spots) on the surface of the kinorhynch body.

Comparison within Kinorhyncha

The discussion above has revealed several variations among features of the nervous system in Kinorhyncha. The number of nerve strings in the ventral cord varies from four in *A. paulae* and *Z. brightae* to three in *E. spinifurca* by a joining of the two central neurites in the latter. While *E. spinifurca* shows an apparent ganglionated or segmental pattern of somata in every segment of the ventral nerve cord, they are sparsely scattered without a clear segmental pattern in *A. paulae* and *Z. brightae*. Because serotonin is hypothesized to be the primary myoexcitatory neurotransmitter, these differences may relate to the type and arrangement of the trunk musculature, which is strongly segmented in *Echinoderes* (Kristensen and Higgins, 1991) and *Antygomonas* (Müller and Schmidt-Rhaesa, 2003). The cuticle is distinctly thicker and more sclerotized in species of *Echinoderes* than it is in *Zelinkaderes* and *Antygomonas*. Functionally, the presence of a sclerotized cuticle in *E. spinifurca* agrees with its notable segmentation, which enables movements of an otherwise rigid trunk. This, in turn, involves the presence of strong, specific musculature that requires adequate attachment points (thick cuticle) and therefore is also arranged segmentally. Consequently, the innervation of this musculature may appear more condensed and segmentally specialized. Regarding their nervous system, homalorhagid kinorhynchs have been poorly studied (Schmidt-Rhaesa, 2007), although their thick sclerotized cuticle and segmented muscular system (Rothe and Schmidt-Rhaesa, 2004) generally support this line of reasoning.

Nebelsick (1993) described the ventral nerve cord terminating in a caudal ganglion in *E. capitatus*. In *E. spinifurca*, there is a pair of big ventrolateral cell somata on segment 10 with strong serotonin-like IR, while in *A. paulae* and *Z. brightae* a conspicuous neurite loop occupies the last two segments. It is difficult to unequivocally correlate the two different structures reported here with the caudal ganglion of Nebelsick (1993). However, both functional and evolutionary implications can be suggested. The big ventrolateral somata and the neurite loop are located very close to the gonopores, suggesting a relationship with the control of muscles involved in reproduction. Due to cutting of the posterior end for immunolabeling in some of the studied specimens, it was not possible to identify them as males or females. Nevertheless, all of the specimens under investigation showed either the large posterior neural somata or the neurite loops described previously, and therefore we assume that they exist in both sexes. Zelinka (1928) noted similar structures for *P. communis* and Higgins (1961) described that an enlarged posterior region of the double ventral nerve cord was present only in females. Kristensen and Higgins (1991) suggest the possibility that such structures have some special function, perhaps in secretory control for the deposition of eggs. Another possible function for these terminal structures related to

reproduction in females could be to control the muscular contraction needed to squeeze the female seminal receptacles at the moment of fertilization.

Regarding the presence of these neural structures in male individuals, it should be noted that the so-called penile spines are not connected with muscles, although their flexible structure is assumed to have a sensory function (Neuhaus, 1999; Neuhaus and Higgins, 2002). Therefore, in parallel with the role suggested above for females, these terminal nerve structures may be involved in the muscular control for the discharge of sperm. Thus far, specific muscles involved in this process in either males or females have not been described as part of the kinorhynch musculature (Müller and Schmidt-Rhaesa, 2003; Rothe and Schmidt-Rhaesa, 2004; Schmidt-Rhaesa and Rothe, 2006).

Alternatively, there is another function unrelated to reproduction that can be suggested. The ventrolateral large cell somata and loop could be involved in the muscular control of the highly movable terminal spines of kinorhynchs (laterotermінal spines, laterotermінal accessory spines, and the midterminal spine). Each type of spine has strong musculature associated with it, at least in specimens of *Antygomonas* (Müller and Schmidt-Rhaesa, 2003). In this respect, it is very interesting that a terminal loop appears only in species having a midterminal spine, such as *A. paulae* and *Z. brightae*, while the paired lateroventral aggregations have been found only in species lacking a midterminal spine, namely, *E. spinifurca*. Juvenile stages of both Cyclorhagid and Homalorhagid species have a midterminal spine that does not remain in their respective adult stages. According to Neuhaus (1993), the possession of a midterminal spine during postembryonic development represents a plesiomorphic condition within Kinorhyncha. Therefore, the loss of a midterminal spine may have occurred independently during evolution in two unrelated families, Echinoderidae (Cyclorhagida) and Pycnophyidae (Homalorhagida). This hypothesis should be tested using a more comprehensive phylogenetic framework of relationships within the phylum.

Comparison with Closely Related Groups

Kinorhyncha is most closely related to the groups Loricifera and Priapulida, which are often collectively referred to as Scalidophora (Lemburg, 1995). Kinorhyncha, Loricifera, and Priapulida (only in Tubiluchidae) share a tripartite brain with anterior and posterior portions of neuronal somata separated by the centrally located neuropil, a feature that was likely inherited from their most recent common ancestor (Rothe and Schmidt-Rhaesa, 2010). A longitudinal arrangement of the brain somata into distinct radial clusters can also be recognized in close association with the first row of spines and introvert retractors: 10 lobes in Kinorhyncha, 8–10 in Loricifera, and at least eight in Priapulida (Kristensen and Higgins, 1991; Kristensen, 1991; Rothe and Schmidt-Rhaesa, 2010). With respect to the epidermis, the position of the brain lies terminally between the anteriormost ring of introvert appendages and the mouth cone (Nebelsick, 1993), and it is flanked by the introvert and mouth cone retractor muscles (Neuhaus, 1994; Nielsen, 2012), a position that is common among Kinorhyncha, Loricifera, and Priapulida. However, a detailed examination suggests that the ring structure of the brain could be derived from a bilateral arrangement. The aggregation of anterior neuronal somata is not a complete ring but is horseshoe shaped, as advanced by Nebelsick (1993) and confirmed by our observations. It is clear that the circular or ring-like arrangement of the brain also corresponds to the circular pattern of introvert appendages or scalids, which show an arrangement of up to seven circles in variable numbers (Zelinka, 1928; Higgins, 1961; Kristensen and Higgins, 1991; Sørensen and Pardos, 2008). Such a circular arrangement does not exhibit a strict radial symmetry, but consistently shows elements suggesting an ancestral bilateral symmetry, particularly in the posteriormost rows or circles of scalids and trichoscalids. Environmental constraints from the intrabenthic, three-dimensional habitat of these animals may have lead to the development of this “pseudo-radial” arrangement. Typically, bilateral elements of the introvert appendages are most clearly seen along the midventral line, a position corresponding to the point where the nerve rings of the brain are open or incomplete. Such bilateral elements are also present in other Scalidophorans, namely in the members of Loricifera, where specialized scalids constitute the so-called “double organ” (Kristensen, 1983; Higgins and Kristensen, 1986). And paired midventral scalids “break” the otherwise radial symmetry of the introvert in at least five genera and species of priapulids (Adrianov and Malakhov, 1999, 2001). Additional information on the brain structure of loriciferans (Kristensen, 1991) and priapulids (Storch, 1991; Schmidt-Rhaesa, 2010) show a more complete, or perfect ring structure, suggesting that kinorhynchs represent the most plesiomorphic bilateral arrangement as far as the brain structure is concerned.

The presence of a partially or semi-paired ventral nerve cord in Kinorhyncha was previously reported by Nebelsick (1993) and our results for the genus *Echinoderes* agree with this statement. In priapulids, the ventral nerve cord is unpaired along its length while in loriciferans it is distinctly paired (Kristensen, 1991). Rothe and Schmidt-Rhaesa (2010) assigned a derived apomorphic state for the unpaired ventral nerve cord of priapulids within Scalidophora, implying that a paired

ventral nerve cord represents the plesiomorphic state.

Regarding the segmental organization of the ventral nerve cord, loriciferans and most kinorhynchs show a ganglion-like clustered pattern, while priapulids do not show any such pattern. In priapulids and loriciferans, neuronal somata associated with the ventral nerve cord are scarce in the anteriormost trunk region, and may indicate an adaptation of the nervous system to mechanical stress associated with retraction and eversion of the introvert (Kristensen, 1991; Rothe and Schmidt-Rhaesa, 2010). Within kinorhynchs, this was previously reported for *E. capitatus* by Nebelsick (1993). We have not only confirmed this statement, but also show the precise location where four strings become three by the fusion of the two midventral strings within the first segment (see Results and Figs. 5, 6). This makes sense if, as suggested above, the location of fusion is where the ventral cord is firmly attached to the body wall. Anterior to this site, the cord is an apparently flexible bundle of neurites extending from the second incomplete ring that varies in position during introvert movements (Fig. 7).

The existence of a caudal ganglion has been previously reported in all scalidophorans: Loricifera (Kristensen, 1991; Malakhov and Adrianov 1995), Kinorhyncha (Kristensen and Higgins, 1991; Nebelsick, 1993), and Priapulida (Rothe and Schmidt-Rhaesa, 2010). We observed either anti-5-HT-like IR cell somata or a neurite loop within segments 10–11, which are two distinct terminal structures. The presence of each structure is consistent between two well-defined kinorhynch groups both without and with a midterminal spine, respectively, and the possible functional significance of each structure in relation to the process of reproduction and/or movement of the terminal spines has been discussed above. A posterior swelling of the ventral longitudinal nerve cord showing high anti-5-HT-like IR has been reported at the base of the tail in several species of priapulids and was considered to be the origin for innervation of the caudal appendage, which is absent in nontailed priapulids (Rothe and Schmidt-Rhaesa, 2010). Similarly, we found a terminal neurite loop in species of kinorhynchs having a midterminal spine, while those species without such a spine also lack the terminal loop. Although a phylogenetic value has not been assessed for the priapulid tail, we hypothesize that the presence of a terminal neurite loop is a plesiomorphic feature within scalidophorans, and may be correlated with the occurrence of a terminal structure (midterminal spine, caudal appendage). To date, no immunolabeling data on the nervous system of loriciferans are available. Remarkably, loriciferan larvae have caudal appendages, the so-called toes that are absent in adults. A comparative study of caudal nerve structures in both larval and adult loriciferans is required to support or reject the plesiomorphic character status for a caudal nerve loop in Scalidophora.

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Appendix II. New neuroanatomical data of cyclorhagid kinorhynchs through immunocytochemistry and CLSM.

This Appendix gathers additional unpublished data from new experiments on the nervous system of kinorhynchs. These experiments were carried out in the same three genera and species of the previous studies (*Echinoderes*, *Antygomonas* and *Zelinkaderes*) but using for the first time anti-alpha tubulin labeling, for axonal processes, combined with propidium iodide, for DNA, and phalloidin for muscles (Figs A.II. 1-4). These novel results complement and widen the results obtained in the first immunocytochemical study of Chapter VII providing new insights in the structure of the nervous system in cyclorhagid kinorhynchs. These results are part of a broader on-going investigation and are meant to show the improvements and potential of the application of immunocytochemical techniques in Kinorhynchs, therefore they will not be described and discussed in depth.

The combination of different markers allowed the better localization and identification of the nerve fivers and their relation with the different organ systems. Overall, results show that the nervous system is composed of a brain divided into three regions with anterior (ans) and a posterior (pns) aggregations of somata from the neurons, and a central neuropil from which arise the ventral nerve cord and four additional longitudinal neurite bundles that interconnect through commissures (normally two per segment) (Figs A.II. 1-2). The ventral nerve cord branches out posteriorly from segment 9 to innervate the posteriormost segments and the associated spines breaking the segmental arrangement showed in previous trunk segments (Fig. A.II. 1, 4B). The comissures and the lateral longitudinal neurite bundles seem to innervate the musculature of the trunk (Fig. A.II. 4 A-B).Each sensory spot seems to be innervated by two long neurites arising either from the comissures or the connectives (Fig. A.II. 4). The introvert and the mouth cone are highly innervated from the neuropil, showing neurites inside each scalid and outer oral style (Fig. A.II. 2). The pharynx is as well innervated and connected with the neuropil in the three genera (Fig. A.II. 2C, 3C). In *Echinoderes* each primary spinoscalid of the introvert seems to be innervated by two neurites, while remaining scalids are only innervated by one neurite each (Fig. A.II. 2B).

Major differences among *Echinoderes*, *Antygomonas* and *Zelinkaderes* nervous system architecture are in the composition of the neuropil and the distribution of the ventral nerve cord associated somata that might be important in order to interpret the evolution of characters within the phylum and within Cycloneuralia and by extension Ecdysozoa (Fig A.II. 3). In *Echinoderes* distinct ganglia can be detected along the ventral nerve cord but they only seem to be present until segment 9 (Fig. A.II. 1B, C), whereas in *Antygomonas* and *Zelinkaderes* the ganglia are not well defined and the somata are distributed along the ventral nerve cord not following a clear segmental pattern (Fig. A.II. 3D-F). The neuropil is ring-like in the three genera under study, but its width and cofiguration seems to change especially in *Zelinkaderes* (Fig. A.II. 3 A-C). Further studies combining different markers are needed in order to obtain more details of the organization of the nervous system in different species and genera.

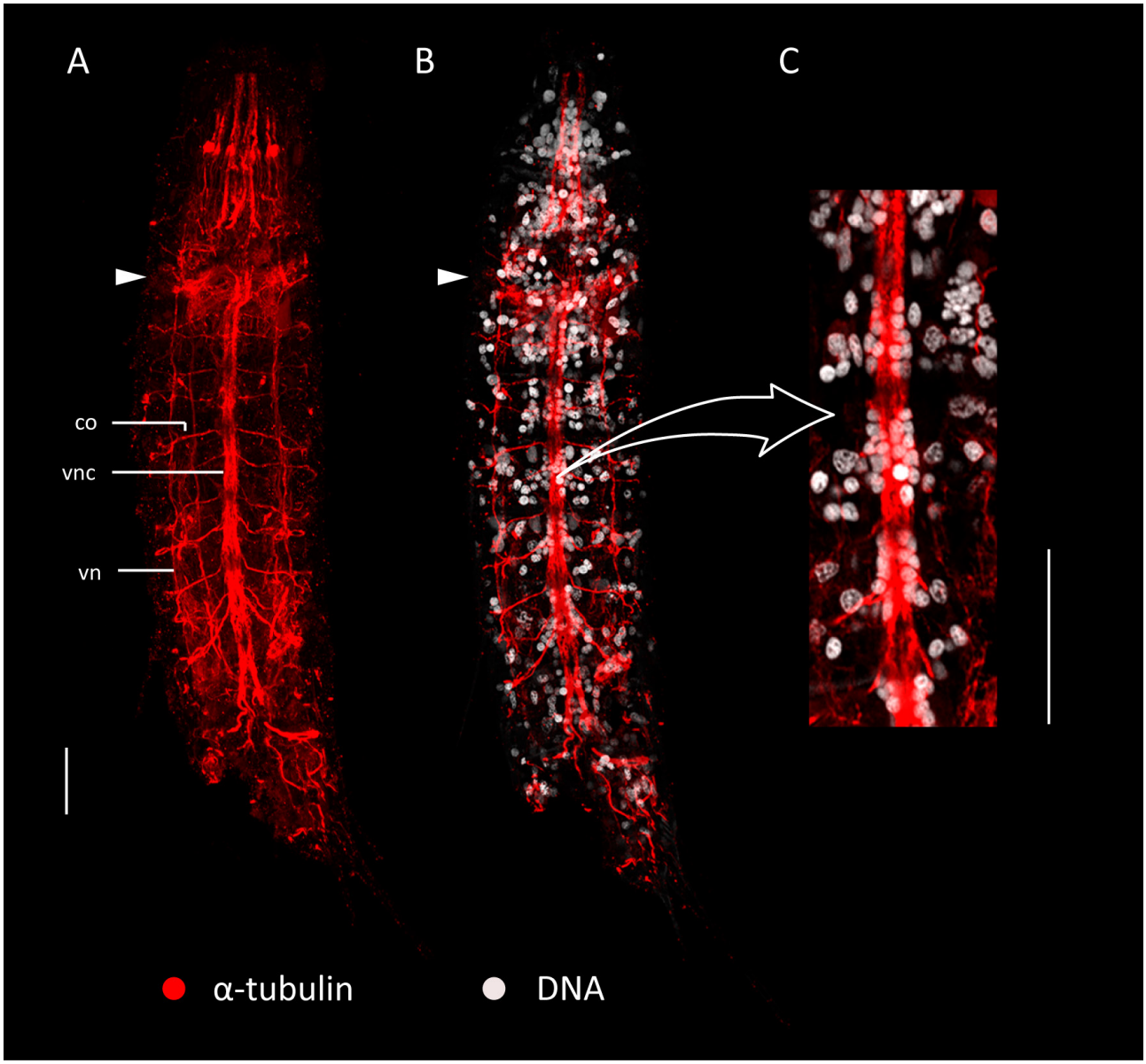

Fig. A.II. 1. Organization of the nervous system in *Echinoderes* as revealed by anti-acetylated α-tubulin immunolabeling and Propidium iodide (DNA) labeling. The images are confocal z-stack projections of *Echinoderes spinifurca*, anterior is up. (A) Anti-acetylated α-tubulin immunoreactivity showing the ventral nerve cord and four additional neurite bundles connected through commissures, ventral view, introvert and mouth cone retracted. (B) Anti-acetylated α-tubulin immunoreactivity combined with DNA labeling, ventral view. (C) Anti-acetylated α-tubulin immunoreactivity combined with DNA labeling of a region of the ventral nerve cord, distinct groups of neuronal somata are detectable each one corresponding with a segment. Abbreviations: co, commissure; vn, ventral neurite bundle; vnc, ventral nerve cord. Scale bars: 20 µm.

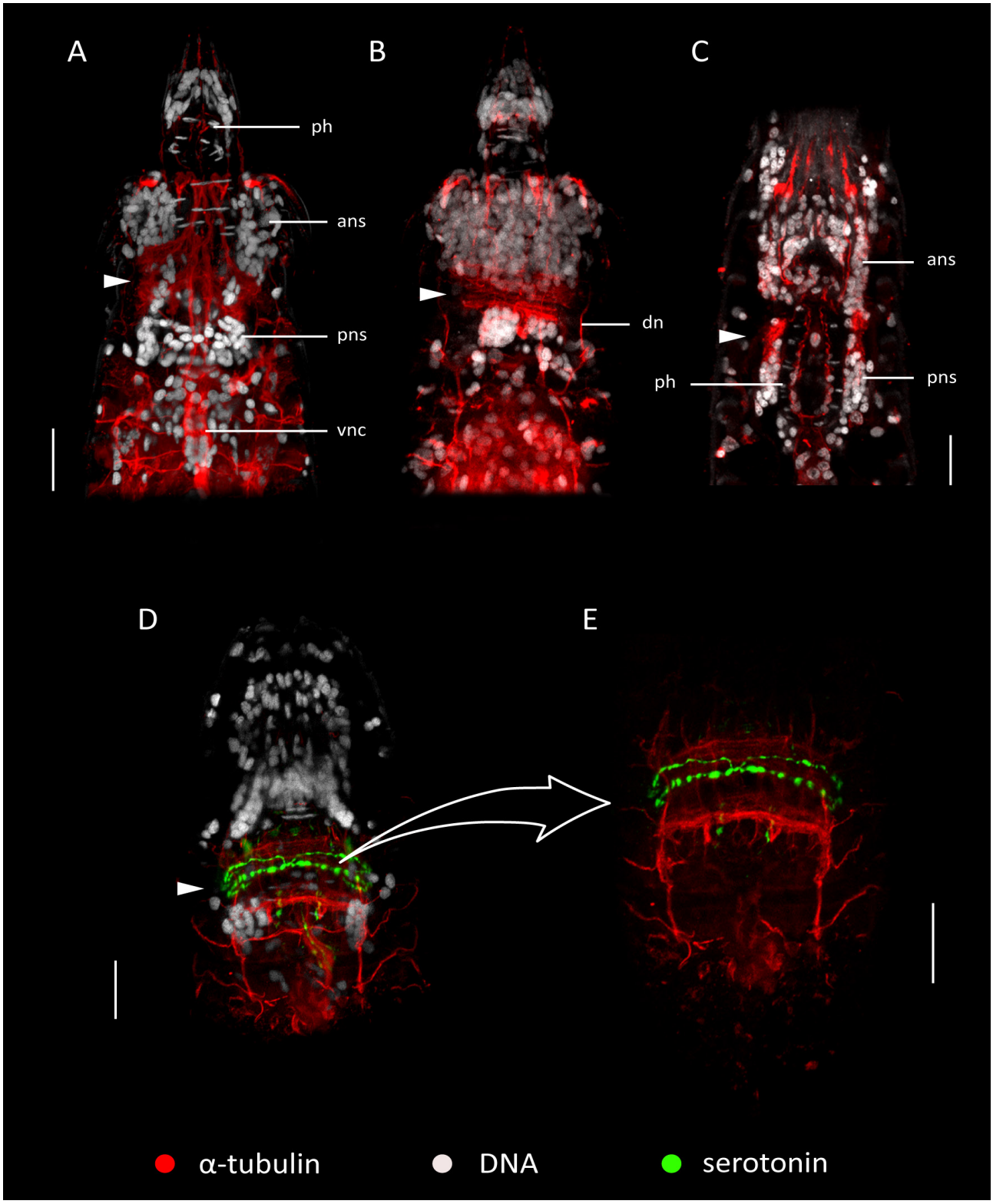

Fig. A.II. 2. Organization of the brain in *Echinoderes* revealed by DNA labeling (Propidium Iodide), anti-acetylated α-tubulin and anti-serotonin-like immunoreactivity. Confocal z-stack projections of the anterior region of *Echinoderes spinifurca,* anterior is up. The brain is regionally divided into two neural somata aggregations separated by a central neuropil. (A) Introvert and mouth cone everted, ventral view. (B) Introvert and mouth cone everted, dorsal view. (C) Introvert and mouth cone retracted, pharynx level, ventral view. (D) Introvert and mouth cone retracted, dorsal view. (E) Detail of the neuropil combining anti-acetylated α-tubulin and anti-serotonin-like immunoreactivity, dorsal view. Abbreviations: ans, anterior neuronal somata; dn, dorsal neurite bundle; ph, pharynx; pns, posterior neuronal somata; vnc, ventral nerve cord. Arrow head indicates the position of the central neuropil. Scale bars: 20 µm.

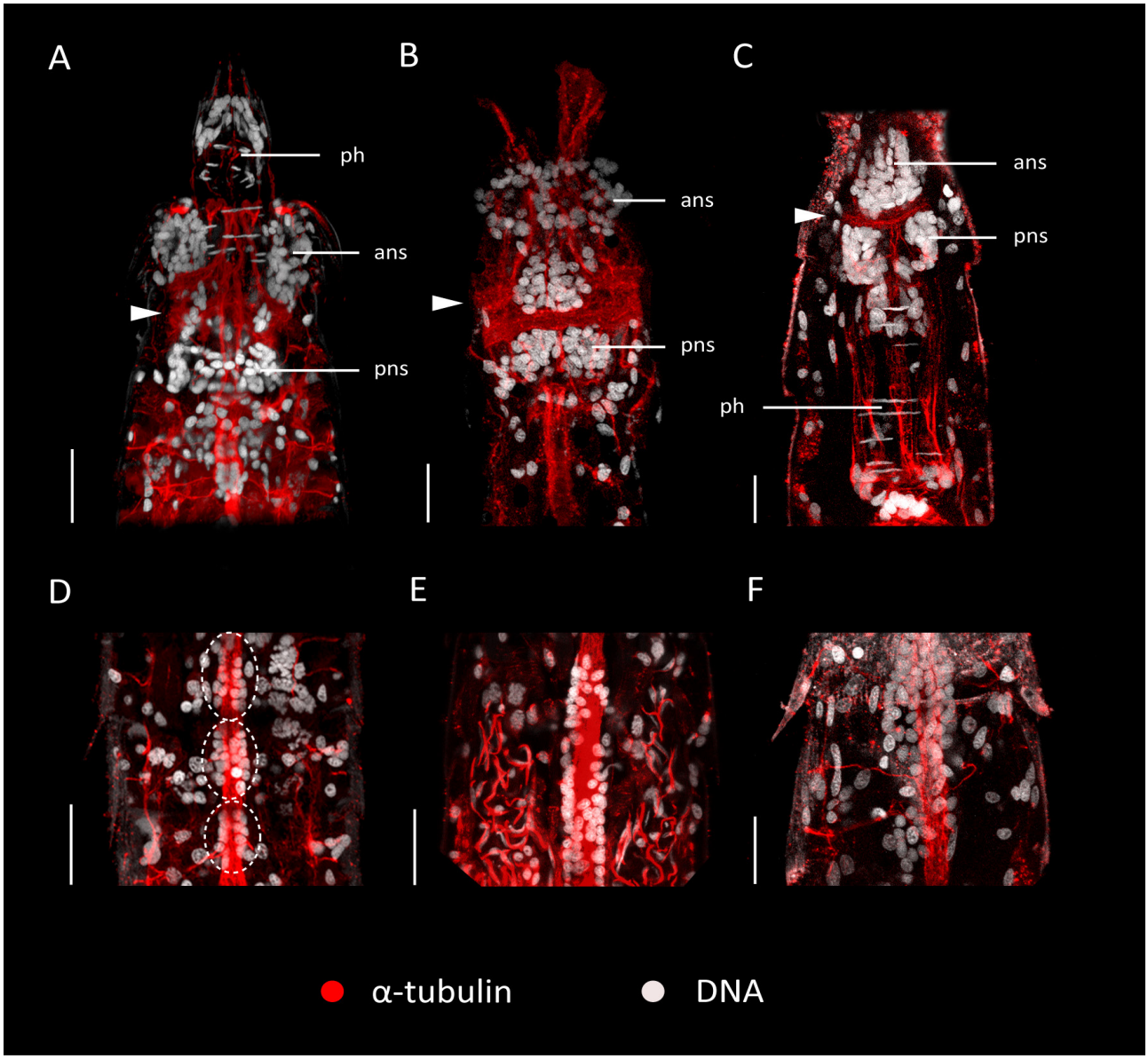

Fig. A.II. 3. Comparison of the brain and ventral nerve cord in *Echinoderes*, *Antygomonas* and *Zelinkaderes* revealed by anti-acetylated α-tubulin immunolabeling and DNA labeling. Confocal z-stack projections of (A, D) *Echinoderes spinifurca*, (B, E) *Antygomonas paulae*, and (C, F) *Zelinkaderes brightae*, ventral views with anterior to the top in all images. (A) Anterior region of *Echinoderes*, introvert and mouth cone everted. (B) Anterior region of *Antygomonas*, introvert partially everted, mouth cone retracted. Note the innervation of the introvert scalids and the mouth cone styles. (C) Anterior region of *Zelinkaderes*, introvert and mouth cone retracted. Note the innervation of the pharynx. (D) Detail of the ventral nerve cord in *Echinoderes*, distinct groups of neuronal somata (dashed circles) are present in each segment. (E, F) Detail of the ventral nerve cord in *Antygomonas* (E) and *Zelinkaderes* (F), somata associated with the ventral nerve cord are abundant but without a clear segmental pattern. Wavy structures labeled at both sides of the ventral nerve cord in (E) correspond to sperm which flagella contain tubulin. Abbreviations: ans, anterior neuronal somata; ph, pharynx; pns, posterior neuronal somata. Arrow head indicates the position of the central neuropil. Scale bars: 20 µm.

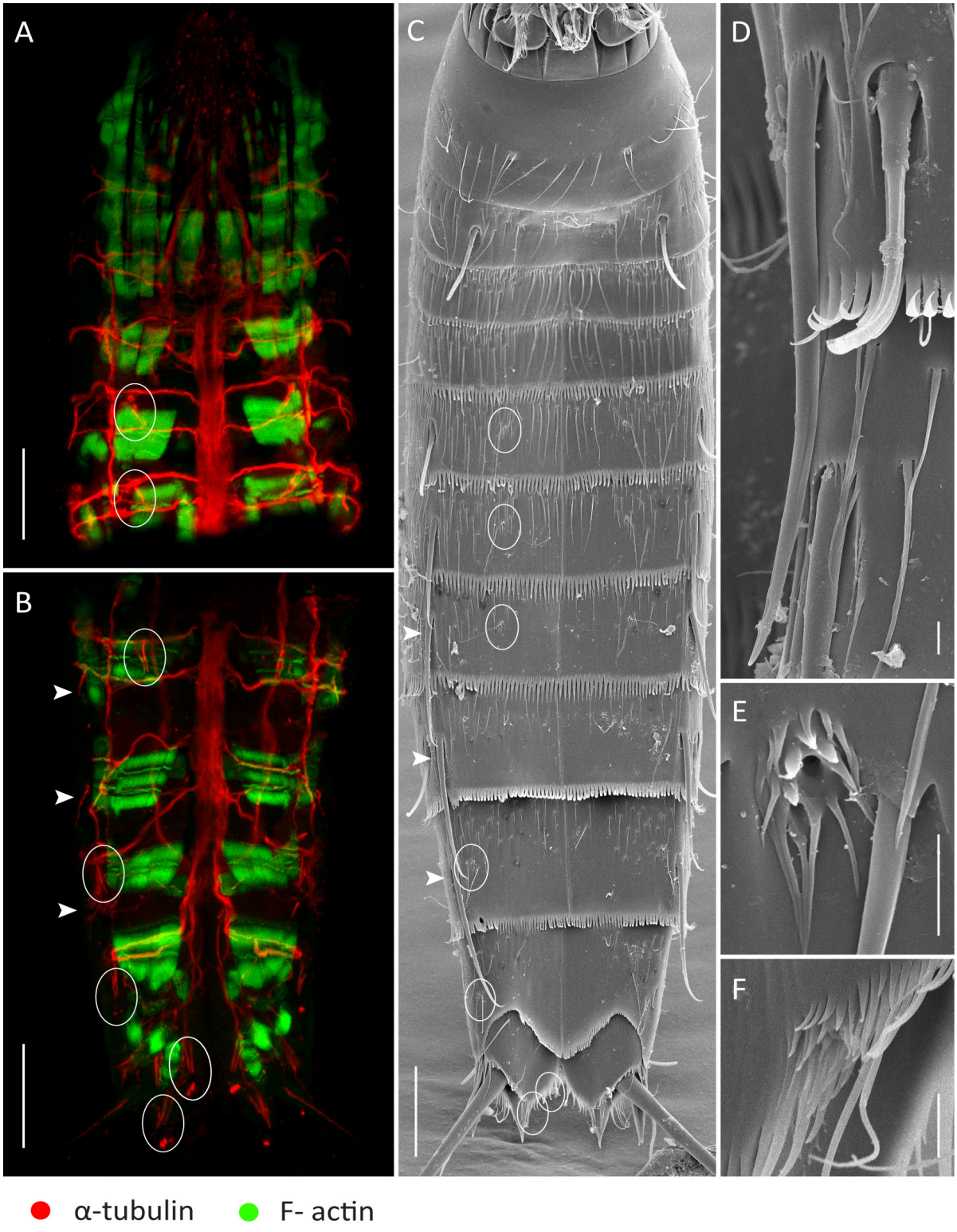

Fig. A.II. 4. Nervous and muscular architecture in *Echinoderes horni*. (A, B) Anti-acetylated α-tubulin immunolabeling and F-actin labeling. (C-F) Scanning Electron Microscopy (SEM). Ventral views, with anterior to the top in all images. (A) Confocal z-stack projection of the anterior trunk region (segments 1-6), introvert and mouth cone retracted. (B) Confocal z-stack projection of the posterior trunk region (segments 7-11). Note that the ventral nerve cord diverge posteriorly from segment 9 to innervate the terminal spines and sensory areas**.** (C) *Echinoderes horni* introvert and mouth cone retracted. The comparison side by side of figures A and B with figure C allow the identification and location of the cuticular structures innervated such as spines and tubules. Sensory spots always innervated through two neurites. (E) Detail of spine and tubule of segment 8. (F) Detail of a sensory spot of segment 9, note the presence of two pores corresponding with the two neurites observed in the position of each sensory spot in A-B. Scale bars A-C: 20 µm, D-F: 1 µm.

The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'

Isaac Asimov

4 General discussion

The investigations carried out in the framework of the present thesis provided new biogeographical, taxonomical and morphological data that somehow contributed to fill some of the gaps pointed out in the introduction, but also brought up new topics and questions.

4.1. Is the Iberian Peninsula a kinorhynch hotspot?

As reported in the introduction, prior to our research the studies on kinorhynchs in the Iberian Peninsula were scarce and merely reduced to a couple of reports and few descriptions. The biogeographical study of Chapter I yielded 29 species, 18 of them cyclorhagids. Within the cyclorhagids a new genus (*Meristoderes*), described in Chapter II, and five new species were found, one of them (*Dracoderes gallaicus*) described in Chapter III. Additional samplings in the Iberian coast (Figs A.I. 1-2, Table A.I. 1) added 7 new still undescribed species to the previous list, most of them belonging to the genus *Echinoderes*. In total, more than half of the current cyclorhagid genera (9 out of 17) and 24 species were reported along the Iberian coasts. It is worth pointing out that the majority of these results come from samplings performed in shallow waters (less than 50 m depth). Therefore, it seems very likely that extensive samplings in deeper waters can unveil an even higher diversity. Regarding the obtained and potential data it would be tempting to designate the Iberian Peninsula as a new kinorhynch hot spot. However, this diversity could just be the result of an intensive sampling effort. Comparable investigations have been carried out in some Mediterranean areas, the coasts of Korea, and the East coast of the United States (e. g. Zelinka 1928; Karling 1954; Higgins 1960, 1961, 1964, 1977, 1978, 1986, 1990; Nebelsick 1990; Sørensen et al. 2005, 2007, 2012; Sørensen 2007) yielding also a high diversity of kinorhynchs similar with what we observe in the Iberian Peninsula (see Neuhaus 2013). Consequently, with the available data and until more areas are studied with similar sampling efforts, it would not be adequate to consider the Iberian Peninsula a hot spot for kinorhynch fauna but just a well sampled area.

4.2. Kinorhynch diversity along the Atlantic vs. Mediterranean coasts of the Iberian Peninsula

The Iberian Peninsula is an especially interesting area for the study of kinorhynchs due to its singular location in between the Atlantic Ocean and the Mediterranean Sea which provides an ideal situation for biogeographical and ecological studies. The results obtained from the biogeographical study do not show significant differences in taxon diversity among the fauna of the Atlantic vs. Mediterranean waters (Chapter I). However, our samples are biased towards Atlantic localities and the results might not be very conclusive until more extensive sampling coverage is accomplished in Mediterranean localities. Additionally, the biological and geographical frontier between the Atlantic Ocean and the Mediterranean Sea does not seem to be coincident. Most southern "Mediterranean" localities selected for our study such as Málaga, Almuñécar and Almería have a big influence of Atlantic waters which enter the Mediterranean through a strong surface current. Therefore their kinorhynch fauna is more similar with the fauna described from Atlantic localities rather than from Mediterranean ones (Chapter I, Appendix I). Several phylogeographic studies in different marine organisms seem to establish the biological limit between both Seas in the Almería-Oran Font rather than in the strait of Gibraltar being the area between both a transition zone (Patarnello 2007). However, such studies are not focused on the benthic realm and their conclusions should be taken with caution regarding the interstitial habitats. Our results show that only one species, *Echinoderes cantabricus* could exemplify the Atlantic influence on Mediterranean waters through its distribution. This species is widely distributed in Atlantic coasts but is only present in the mentioned mediterranean localities with high influx of Atlantic waters. However, Atlantic influence is mostly superficial and we do not know how it can affect neither the deep dispersion patterns nor the distribution of kinorhynch populations in deeper waters.

Kinorhynch species with a wide distribution in both Mediterranean and Atlantic localities such as *Centroderes spinosus*, *Echinoderes dujardinii, Echinoderes hispanicus, Echinoderes* sp.1 and *Semnoderes armiger* suggest a colonization process from Atlantic waters. From them, *Semnoderes armiger* was also found in Naples coasts suggesting a wide distribution in both Atlantic and Mediterranean waters (Fig. A.I. 2). *Centroderes spinosus*, *Echinoderes dujardinii* and *Semnoderes armiger* have also been reported over time in different localities from the North Sea (Atlantic) to the Black Sea (Mediterranean) confirming the wide distribution found in our studies. Contrarily, we could also find exclusive species from Mediterranean localities with a wide distribution such as *Antygomonas incomitata*, a typical Mediterranean species (Nebelsick 1993; Sørensen et al. 2009; Sørensen et al. 2010a), and *Meristoderes macracanthus* present in Blanes, Sardinia and Naples (Fig. A.I. 2). Other species appear to have restricted distributions sometimes found in a single or very few localities such as the new *Echinoderes* sp.3 -5 and *Sphenoderes* sp. (Fig. A.I. 2). Future samplings will be necessary in order to consider them as having restricted distributions or just being the reflection of local findings.

4.3. The paradox of Kinorhynch distribution

Kinorhynchs are part of the permanent meiofauna, meaning that they spend their entire life cycle in the sediment. They are direct developers, lacking larval planktonic stages and thus being assumed to have a limited dispersive potential (Higgins and Thiel 1988; Giere 2009). Moreover, and as many other meiofaunal groups, kinorhynchs are equipped with mucous glands and cuticular structures (spines and tubules) that enable them to be attached to the sediment, preventing accidental detachments that could bring them into the water column and drift (Brown 1989). However, the wide distribution showed by some genera and species leads

to a paradox. The mechanisms of dispersion underlying these distributional patterns are not conclusive and still remain debated. However, potential pathways through deep bottom currents have been addressed for *Campyloderes* which shows a ubiquitous distribution (Neuhaus and Sørensen 2012). Vicariance processes through continental drift have also been suggested to explain the amphioceanic distribution of new species of *Centroderes* (Neuhaus et al. in press).

Some distribution patterns seem to be related to geological events such as the formation of the current Mediterranean Sea. This is the case of two species of *Meristoderes: M. macracanthus* from the Mediterranean Sea and *M. boylei* from the Atlantic coast of Florida. As discussed in Chapter V both species have a striking morphological similarity despite their distant distribution areas. It seems very likely that both species could have diverged from the same Atlantic ancestor and evolved independently in each area following allopatric speciation. This could have occurred after the Messinian Crisis, when the Mediterranean Sea was colonized by Atlantic marine fauna through the opening of the Gibraltar Strait (García-Castellanos et al. 2009). The high resemblance of both species could be explained by a low speciation rate. The very fast recolonization of the Mediterranean Sea might also explain the distribution of other Atlantic species present in Mediterranean coasts such as *Semnoderes armiger* or *Centroderes spinosus* (Fig. A.I. 2).

Disjunct distributions are present for the enigmatic genus *Dracoderes*, which was apparently restricted to Japanese waters (Higgins and Shirayama 1990) but has been frequently found in the Atlantic and Mediterranean coasts of the Iberian Peninsula (Chapter III, Fig. A.I. 2). Our guess is that this genus will appear in other areas between the Iberian Peninsula and Japan, therefore the current distribution is reflecting once more an incomplete sampling. Apart from *Dracoderes* other genera such as *Fissuroderes* and *Meristoderes* appeared to have disjunct distributions that seem to be an artifact of punctual findings stressing the need of more extensive samplings.

Recent phylogeographic studies focused on other meiofaunal groups lacking dispersal stages, such as gastrotrichs (Kieneke 2012), suggest that some species considered as ubiquitous were in fact formed by a group of several cryptic species with restricted distributions. Following this promising path, phylogeographical investigations would be recommendable to shed light into the paradox of the kinorhynch distribution.

Locally the distribution of some kinorhynchs seems to be highly associated to certain types of sediments and depth. Although rigorous ecological studies have not been carried out in the Iberian Peninsula, we could detect clear preferences for types of sediment by some species (Chapter I) such as *Dracoderes gallaicus,* which was always associated with very fine and viscous mud, and species of *Antygomonas* normally associated with sandy or coarse sediments. These results are based on subjective categories because no standard analytical granulometric tests could be applied to all sediment samples and therefore are only tentative. Apparent depth associations could be an artifact of the sampling mostly carried out in shallow waters.

4.4. Which is the estimated global diversity for kinorhynchs?

Kinorhynchs are considered one of the least-known taxonomic groups. It is estimated that less than a 20% of the total species diversity has been investigated (Appeltans et al. 2002). Almost every sampling carried out on the continental shelf or in the deep sea reveals new species for the phylum (Neuhaus and Blasche 2006; Sørensen 2006, 2007, 2008a; Sørensen et al., 2000, 2007, 2009, 2010a, b, c, 2012, 2013; Sørensen and Rho 2009; Sørensen and Thormar 2010; Dal Zotto et al. 2013; Sánchez et al. 2014). Therefore, at least 1.000-2.000 species of Kinorhyncha can be expected to live in marine environments (Appeltans et al. 2002). During the

last four decades the description rate of kinorhynchs have been increasing constantly, this may be due to a more taxonomic effort, new technologies, exploration of new habitats and localities, use of molecular methods, or a combination of all these factors.

The current lack of knowledge of the diversity of kinorhynchs warns us about the still limited sampling and the need of extensive collections in the phylum. Even results of the samplings carried out in relatively well studied areas, such as Florida coastal waters and the Bay of Naples, have provided new species and recordings (Appendix I: Figs A.I. 3-4, Tables A.I. 4-5). Floridian coasts have been multiple times sampled over the years by R.P. Higgins and M.V. Sørensen (Higgins 1990; Sørensen et al. 2005; 2007) but new collections in 2011 (Appendix I: Fig. A.I. 4, Table A.I. 3) reported 6 new species that were described in Chapters IV and V. The same happened in Naples where old Zelinka's samplings were reproduced after almost 100 years, yielding as well several new species and reports for the area that are still under study (Appendix I: Fig. A. I. 3, Table A.I. 2). This shows that even relatively well studied areas still hold undiscovered kinorhynch diversity, and that over time kinorhynch fauna can experience changes in a region. So far there are not studies focused on the dynamic of kinorhynch populations through a prolonged time in the same area. But the ongoing studies in Naples suggest that some species with a reported wide distribution such as *Echinoderes dujardinii* or *Echinoderes capitatus* (Zelinka 1928; Higgins 1983) could now be reduced and displaced by other species.

4.5. Taxonomic investigations on cyclorhagid kinorhynchs

All the samplings carried out in the different investigated areas (Materials and methods section) yielded 1 new genus and 25 new species for the science, 8 of these species were described in Chapters II-V, whereas the remaining 17 still await formal description. The described species belong to 5 different genera, four of them containing less than 5 species, consequently the contribution to the knowledge of these genera was significant. This contribution was possible due to the combination of LM and SEM techniques which allowed a better understanding and accuracy of the descriptions of the new taxa. Many cuticular structures, not previously visualized and overlooked with LM, were discovered with SEM becoming relevant at taxonomic and phylogenetic levels.

Genus Dracoderes (Chapter III)

The redescription of the type species *D. abei* was possible through the SEM study of new specimens from Korea and Japan waters that were considered conspecific with *D. abei*. Additionally, a new species from Atlantic waters, *D. gallaicus,* was described combining SEM and LM observations. The use of SEM in the mentioned species allowed the discovery of small tubules and detailed observations of the lateroventral spines, as well as the mapping of the sensory spots, which provided new differential characters to help in the identification of otherwise similar species of the genus. The visualization of the nephridial pores, the introvert, and mouth cone for the first time provided as well multiple details never shown before in the genus. The outer oral styles showed conspicuous alternating sizes, this together with the special appearance of the nephridial pores and the reduced number of neck placids, indicated an affinity with other non-echinoderid cyclorhagids and even homalorhagids. This leaded *Dracoderes* to become a key taxon in studies of kinorhynch evolution and stressed the importance of the taxon in phylogenetic studies. In fact, recent phylogenetic studies nested *Dracoderes* out of Cyclorhagida and as part of Homalorhagida (Yamasaki et al. 2013; Dal Zotto et al. 2014), which could be confirmed by the morphological similarities observed in our study. However, this hypothesis needs to be tested with a broader sampling data.

Genus Meristoderes (Chapters II, V)

The discovery of the genus *Meristoderes* was interesting due to its intermediate position among the two extant genera *Echinoderes* and *Cephalorhyncha*. The key character to identify it was the special composition of segment 2 with two incomplete tergosternal divisions. The condition of the second trunk segment seems to be pivotal for the understanding of internal Echinoderid relationships (Sørensen 2008b), and therefore the discovery of this genus could give some hints to discern the evolution of the second trunk segment within the family and by extension the phylum. However, it would be necessary to include species of *Meristoderes* in next phylogenetic studies in order to determine its position among Echinoderidae.

Genus Antygomonas (Chapter IV)

Antygomonas gwenae is another interesting new species described in the present Thesis which showed a mixture of characters shared with *Antygomonas*, *Semnoderes* and *Sphenoderes* that made its genus assignation problematic. These kinds of situations have been described for other taxa such as *Wollunquaderes majkenae* Sørensen and Thormar, 2010 and the mentioned genus *Meristoderes* (Sørensen and Thormar 2010). In the case of *Antygomonas gwenae* we did not erect a new genus and instead the new species was tentatively assigned to *Antygomonas*, which was the most similar genus, until future phylogenetic analysis confirm or reject it.

Genus Fissuroderes (Chapter V)

Fissuroderes sorenseni was the first record of this genus after its discovery by Neuhaus and Blasche (2006). The descriptions of already known species were based on LM only, thus the description of the new species with SEM provided several new characters never showed before that could be autapomorphies of the genus (Chapter V). These new characters were related to the introvert, mouth cone, cuticular hairs, tubules and glandular openings. However, most of them could not be recognizable through LM in previously described species and therefore comparisons within the genus were not possible. One of the important contributions of this new species was the complete description of an introvert of the genus *Fissuroderes* for the first time, revealing an additional row of scalids not found in any other genera. Other interesting discovery is the presence of oral styles alternating in size, the differences are not as conspicuous as those observed in species of *Dracoderes* but can be important in a phylogenetic context. The presence of these minor differences in the size of the outer oral styles was found as well in species of other cyclorhagid genera such as *Echinoderes riceae* (Chapter IV), *Meristoderes macracanthus* and *M. boylei* (Chapters I, V). It seems that this character can be common in more genera and species, thus it would be recommendable to check it in additional taxa in order to evaluate its taxonomic importance.

4.6. Contributions to the terminology of kinorhynchs

As a result of the thorough examination of specimens for the description of the new taxa, it was necessary to clarify and define new characters found in order to standardize and improve accuracy in future taxonomic descriptions.

The main contributions were:

- The regionalization of the posteriormost part of a segment (Chapter II). New terms such as free flap, ij-line and fringe tips were introduced following the precedent of Gª Ordóñez et al. (2000) who described

the primary and secondary pectinate fringes.

• The description of four subtypes of type 1 sensory spots based on the presence and number of associated cuticular hairs (Chapter II). These varieties were found mostly in Echinoderid species.

• Description of an additional row of scalids in the introvert, the accessory trichoscalids (Chapter IV). Due to the detailed description of the introvert of *Fissuroderes sorenseni* there was found an additional row of scalids, very similar to trichoscalids but lacking a trichoscalid plate. This new variation of the introvert appendages was not reported for any of the known species of the phylum before. However, the first description of additional scalids was the so called leaf-like scalids reported in species of *Meristoderes* and posteriorly described in *Echinoderes* (Sørensen et al., 2013; Chapters II, IV, V). The description and mapping of special types of appendages in the last rows of the introvert is becoming more and more common with the inclusion and improvement of SEM techniques which provides higher optical resolution of surface structures. However, this task is always difficult because the scalids are usually hidden under the scalids from previous rings and therefore they are difficult to see. Unfortunately, to date the absence of enough detailed introvert studies makes difficult the evaluation of these characters in a phylogenetic context.

4.7. Is introvert mapping a useful taxonomic tool?

The beginning of the use of the SEM allowed a better study of the introvert and mouth cone structures showing a big variability at genus and species level and therefore being a potential taxonomic tool. The amount of reliable information about introvert morphology and scalid arrangements is still too limited and scattered across the taxa to make any conclusions, but some patterns start to become evident. A broad range of taxa sometimes represented by homalorhagid and cyclorhagid species show very similar scalid arrangements. Other cyclorhagids such as species of *Zelinkaderes* and *Triodontoderes* have reduced the number of scalid rings whereas species of *Cephalorhyncha*, *Echinoderes* and the new *Fissuroderes* and *Meristoderes* species often shows additional scalids in the posteriormost rows (Higgins 1990; Nebelsick 1993; Bauer-Nebelsick 1996; Sørensen et al. 2007; Sørensen 2008a; Sørensen and Pardos 2008; Sørensen and Rho 2009; Chapters II, IV, V). Although further data from additional species are required to draw any conclusions, the increasing amount of information clearly shows that the introvert morphology could provide new clues about evolutionary pathways within Kinorhyncha.

4.8. Morphology of cyclorhagid kinorhynchs

Numerous data on the external morphology of different genera and species of cyclorhagid kinorhynchs were gathered in Chapters II, III, IV and V using SEM and LM microscopies. The study of the cuticular morphologic characters allowed the description of new taxa and the definition of new terminology, all discussed in previous sections.

Studies focused on the internal anatomy of kinorhynchs have been primarily based on transmission electron microscopy (Brown 1989; Gª Ordóñez et al. 2000; Kristensen and Hay-Schmidt 1989; Kristensen and Higgins 1991; Nebelsick 1993; Neuhaus and Higgins 1994). The emergence of CLSM initiated a new working field providing new perspectives to the study of the internal anatomy in Kinorhynchs in whole mounts. Only three investigations were carried out in kinorhynchs using this technique, all focused in the myoanatomy, of them just one was performed in a cyclorhagid species (*Antygomonas* sp.) (Müller and Schmidt-Rhaesa 2003; Rothe

and Schmidt-Rhaesa 2004; Schmidt-Rhaesa and Rothe 2006). In the present thesis, a comparative study of the musculature and nervous system of some cyclorhagid taxa was carried out combining cytochemical and immunocytochemical techniques in conjunction with CLSM and 3D rendering (Chapters VI and VII). Moreover, additional new unpublished data on the nervous system was provided in the Appendix II (Figs A.II. 1-4).

4.9. Difficulties of staining kinorhynchs. New protocols

Kinorhynchs are challenging animals to work with, not only for their reduced size but also for the presence of a resistant chitinous cuticle that prevents penetration of most chemical reagents. In order to carry on immunocytochemical studies it was necessary to permeabilize the cuticle of these animals with micro-incisions of the posterior end allowing the antibodies (big molecules) to migrate internally with minimum damage. Cytochemical studies using phalloidin conjugated with a fluorescent dye did not need previous perforation of the cuticle due to the smaller size of the molecules that could diffuse well through both the cuticle and body tissues to bind specifically the F-actin fibers. The presence of a reduced body cavity (Kristensen and Higgins 1991) complicated the easy penetration of conjugated antibodies and phalloidin requiring longer incubation periods and higher percentage of detergents to obtain satisfactory results (see materials and methods of Chapters VI and VII for detailed protocols). These mentioned changes and the diary change of the incubation solution avoided the presence of weak staining in the center of the trunk, a very common problem with these animals.

The high autofluorescence of the cuticle together with the non-specific binding of the secondary antibodies to the scalids and some portions of the epidermis complicated as well the detection of the fluorophores signal. However, the autofluorescence of the cuticle was used in combination with the signal of the different fluorescent dyes to guide interpretations of the internal anatomy in each species.

4.10. Complexity and function of the musculature in kinorhynchs

Kinorhynchs are highly flexible animals able to perform multiple movements through the sediment due to the presence of a burrowing introvert and an articulated trunk divided into cuticular plates, all controlled by a complex array of muscles. The presence of a hard exoskeleton has driven kinorhynchs to abandon the basic structure of antagonistic sets of circular and longitudinal body-wall muscles typically found in soft bodied invertebrates (Schmidt-Rhaesa 2007). The continuous layers of body-wall muscles have been replaced by individual, more specific and isolated muscles attached to the exoskeleton through intermediate epidermal cells (Kristensen and Higgins 1991; Schmidt-Rhaesa 2007). Most of the muscles on kinorhynchs are cross striated for rapid contraction associated with feeding and locomotion (Kristensen and Higgins 1991; Schmidt-Rhaesa 2007). As a general rule, all kinorhynchs, both cyclorhagids and homalorhagids, possess segmental and non-segmental muscles with longitudinal, dorsoventral and diagonal orientations; circular muscles are only present in the introvert, neck and midgut regions.

Echinoderes, which is the most specious genus within Kinorhyncha, was selected as a model to carry on a comparative multi-species study of the musculature (Chapter VI). Results showed that the musculature of different species of *Echinoderes* seems to be very conserved contrary to the great variability of cuticular features exhibited. Only highly complex regions such as the pharynx and head show minor differences among species. The burrowing head acts as a locomotory and feeding structure requiring a specialized musculature (Kristensen and Higgins 1991; Neuhaus and Higgins 2002). The mouth cone and the introvert share the pres-

ence of circular and longitudinal muscles arranged radially and functionally correlated. Both parts of the head act independently from each other having indeed independent musculature. The mouth cone is the anteriormost region of the digestive tract and is involved in the food uptake whereas the introvert is related to the locomotion and sensory perception (Brown 1989; Kristensen and Higgins 1991; Nebelsick 1993; Neuhaus 1994). However, a sensorial feeding-related function was described for the inner oral styles of the mouth cone (Brown 1989; Higgins and Kristensen 1991). Each outer oral style of the mouth cone has a pair of short longitudinal muscles allowing independent motion, whereas introvert scalids do not possess intrinsic musculature, which means that they move passively as a whole.

The neck acts as a closing apparatus due to the presence of a strong circular muscle and alternating soft (interplacids) and hard (placids) cuticular elements. Both neck and introvert are functionally linked and interdependent (Higgins and Kristensen 1991; Chapter V). However, while circular muscles of the mouth cone and introvert move along the anterior-posterior axis together with the head extension/retraction, the neck circular muscle maintains a fixed position (Chapter VI).

The trunk has paired sets of longitudinal, dorsoventral and diagonal muscles that enable kinorhynchs to perform a wide range of movements. Diagonal and dorsoventral muscles seem to be segmentally arranged whereas longitudinal muscles can be segmental and non-segmental. Diagonal muscles are only present from segments 1-8, and contribute to lateral movements of the animal (Zelinka 1928; Remane 1929) while posteriormost segments seem to be less flexible. Dorsoventral muscles are present from segment 3 to 10, their contraction contribute to the eversion of the introvert increasing the internal pressure of the reduced body cavity (Kristensen and Higgins 1991). Longitudinal segmentary dorsal and ventral muscles act as antagonists for the more or less pronounced curvature of the trunk, and the presence of continuous longitudinal fibers might contribute to increase the high flexibility of the trunk observed in living specimens.

The musculature associated to the digestive system is not segmented(Neuhaus 1994). The most complex muscle system in *Echinoderes* and probably all other cyclorhagids is the pharynx which is composed of a pharynx bulb encircled by multiple fibers with different but integrated functions. The pharynx bulb is composed of alternate radial and circular muscles fibers as previously reported in many studies (e. g. Zelinka 1928; Kristensen and Higgins 1991; Neuhaus 1994). Their antagonistic contraction enables the pharynx to function as a muscular sucking pump while anterior and posterior sphincters control the entry and exit of particles respectively (Neuhaus 1994; Nielsen 2012). A complex array of longitudinal muscles surrounds the pharynx bulb with different functions for movement control: retractors, protractors and suspensors (for more details see results and discussion sections of Chapter VI).

The detailed description of the myoanatomy in *Echinoderes* served as a model and starting point to study and compare the musculature of other genera and species within the phylum.

Isolated data from previous studies (e.g. Müller and Schmidt-Rhaesa 2006) pointed out that all cyclorhagid Kinorhynchs seem to have similar kinds and arrangements of trunk muscles. However, main and relevant differences are expected in the anteriormost trunk segments and the neck due to the different closing apparatus. Undoubtedly, the first segment (and the second in *Echinoderes* too) are related and engaged with the in-and-out movement of the introvert in the trunk, which also involves the neck. This is the morphofunctional reason why these segments are closed cuticular rings and not divided into tergal and sternal plates. Of course, differences in the muscular system are expected when trunk closure proceeds in different manners (conchorhagid vs. cyclorhagid, not to say the dorsoventral closure of homalorhagids). Moreover, differences also exist in the musculature associated with the terminal spines of the posterior end. The presence of a mid-

terminal spine, not present in *Echinoderes*, determines special muscle arrangements for such highly movable cuticular appendages.

4.11. Do scalidophorans share a similar myoanatomy?

The presence of an eversible radial head is considered an apomorphy to group loriciferans, priapulids and kinorhynchs into Scalidophora (Lemburg 1995) recognized as a basal branch within Ecdysozoa (Aguinaldo et al. 1997; Garey 2001; Mallat and Giribet 2006; Rota-Stabelli et al. 2010). The comprehensive study of the musculature in *Echinoderes* (Chapter VI) allowed a better comparison within Kinorhyncha and contributed to broaden our understanding of how different types and patterns of musculature evolved within Scalidophora, and by extension, Ecdysozoa. However, there are obvious differences in the size and body plans of the three groups. One of the most important differences is the segmented pattern present in the trunk of kinorhynchs but absent in loriciferans and priapulids with oval and vermiform bodies respectively, which is reflected in the arrangement and complexity of their muscles. Consequently, direct comparisons of the myoanatomy among these groups are only possible within their head and neck regions. All three groups show well developed and combined circular and longitudinal muscles within the introvert, however the number and arrangement differs highly in each phylum. The presence of inner and outer retractors in the introvert attaching to the introvert wall on both sides of the brain of loriciferans, priapulids and kinorhynchs was described as another apomorphy for the scalidophorans (Nielsen 2012). The study in *Echinoderes* (Chapter VI) revealed that these retractors could be associated with different organs (pharynx and introvert) in kinorhynchs, not being homologous among phyla. A recent cytochemical study of the myoanatomy in the loriciferan *Nanaloricus* sp. (Neves et al. 2013) could not provide a clear organ-muscle association of these muscles. Moreover, studies in priapulids did not give positional correlations of the retractor muscles relative to the brain (Schmidt-Rhaesa 2013). So it appears that more information is required to confirm spatial relationships between organ-specific retractors and cycloneuralian brain architecture within loriciferans and priapulids. For a more detailed comparison among phyla see discussion section of Chapter VI.

The three phyla (loriciferans, priapulids and kinorhynchs) are burrowers which can explain the development of a radial symmetry in their anterior ends superimposed to the bilateral body plan that could be most likely an adaptation to their burrowing mode of life, involving uniform contact with the sediment. However, more studies concerning additional organs systems are necessary in order to shed light in the relationships among these groups because the musculature might be influenced by strong habitat constraints. Despite the differences in number and arrangement of muscles, the myoanatomy of kinorhynchs is comparatively more similar with loriciferans rather than with priapulids.

4.12. How is the architecture of the nervous system in cyclorhagid kinorhynchs?

The study of the nervous system carried out in Chapter VII was the first successful attempt to use immunocytochemical techniques in kinorhynchs combined with confocal laser scanning microscopy. The whole nervous system cannot be characterized using a single type of fluorescent marker (Rothe and Schmidt-Rhaesa 2009). However, to establish a first step in a working protocol for kinorhynchs it was reasonable to begin with standard neural markers such as antibodies against serotonin molecules. Serotonin is considered the primary myoexcitatory neurotransmitter in several invertebrate groups (Hochberg 2009). Consequently, the positive immunoreactivity found in our results could be most likely related with locomotory processes. Results showed

a subset of the nervous system with high levels of anti-serotonin-like immunoreactivity (anti-5HT-IR) in the brain and the ventral nerve cord in the three studied genera of kinorhynchs (Chapter VII). It seems that part of the anterior neuronal somata of the brain might be involved in the coordination of the introvert movement while the ventral nerve cord might coordinate the movement of the different sets of trunk muscles. Nebelsick (1993) and Neuhaus (1994) described the presence of nerve fibers extending from the mouth cone nerve ring to the pharynx. This innervation could not be detected with anti-5HT labeling in the first study (Chapter VII), however new studies confirm the presence of anti-5HT-like IR inside the pharynx bulb which can be related with the control of the radial and circular muscles involved in feeding (Chapter VI).

The introvert scalids in kinorhynchs are described as locomotory and sensory appendages (Moritz and Storch 1972a, b; Kristensen and Higgins 1991). However, they did not show clear 5HT-like IR in any of the studies suggesting that the scalids are only indirectly controlled for movement. These results are supported by the myoanatomical results obtained in Chapter VI which show that scalids do not contain intrinsic muscles and move passively as a whole during the retraction and extension of the introvert. In contrast, the nine oral styles of the mouth cone do possess muscles (Kristensen and Higgins 1991; Nebelsick 1993; Neuhaus 1994; Chapter VI). Accordingly, anti-5HT-like IR was found in the base of the mouth cone (Chapter VII) which can be related with the presence of mouth cone circular muscles described in Chapter VI and therefore with the control of the movement of the mouth cone. The ventral nerve cord showed a clear segmental pattern of associated somata in *Echinoderes,* but sparsely scattered and without a clear segmental pattern in *Antygomonas* and *Zelinkaderes* species (Chapter VII, Appendix II). Moreover, the ventral nerve cord ends in two ventrolateral cell bodies in *Echinoderes*, and forms a terminal loop in *A. paulae* and *Z. brightae.* The presence of this loop seems to be directly associated with the existence of a midterminal spine which is controlled by strong muscles as showed in the myoanatomical study of Schmidt-Rhaesa (2003) for *Antygomonas* sp.

In order to refine and complement the nervous system study of Chapter VII, new unpublished investigations were carried out using general markers for neural structures co-labeled with phalloidin (muscles) and propidium iodide (DNA), which helped to obtain a more complete insight of the nervous system architecture in kinorhynchs (Appendix II, Figs A.II. 1-4). One of the new markers utilized were antibodies against alpha tubulin, a major component of axonal processes. Results showed a whole complex of neural fibers forming the neuropil of the brain connecting anteriorly with the introvert and mouth cone, and posteriorly with one ventral nerve cord (with paired origin) and four additional longitudinal neurite bundles, being the ventral nerve cord the most prominent (Figs A.II. 1-2). The five nerve cords are connected by two commissures per segment. These results agree with the results of the ultrastructural studies of Kristensen and Higgins (1991), and Nebelsick (1993). However, there are differences in the anterior and posteriormost segments were the apparent “orthogonal pattern” disappears (Figs A.II. 1, 4). As described by Higgins and Kristensen (1991) the ventral nerve cord is segmentally ganglionated in kinorhynchs however, different patterns were observed in *Echinoderes*, *Antygomonas* and *Zelinkaderes* species, being the segmental pattern of the two latter less distinct as previously noticed with anti-serotonin labeling experiments (Fig A.II. 3). These observations are preliminar and need a more comprehensive analysis, but could reflect different degrees of segmentation within kinorhynchs mirrored by the presence vs. absence of a hard cuticle, which can have important phylogenetic relevance within the phylum. However, further studies including and combining additional markers are necessary in order to detect possible patterns that give some hints of the evolution of the nervous system among kinorhynchs.

4.13. Segmentation in kinorhynchs

Since the description of the first kinorhynch as “something intermediate in between a worm and a crustacean” by Felix Dujardin in 1841, the position of the phylum has been controversial as well as the condition of their segmentation. Kinorhyncha, initially positioned in the artificially created Aschelminthes, is now reliably assigned to one of the bilaterian superclades, the Ecdysozoa (Aguinaldo et al. 1997). However, the origin and evolution of the segmentation in Ecdysozoa and in kinorhynchs is still under debate. Kinorhynchs are considered the most basal segmented ecdysozoans and therefore the study of their internal anatomy seems crucial in an evolutionary context (Aguinaldo et al. 1997; Neuhaus and Higgins 2002; Halanych 2004; Sørensen et al. 2008). However, the difficulty of culturing kinorhynchs and the lack of developmental studies do not allow us to have a better understanding of the origin of the segmented organ systems and establish possible homologies with other ecdysozoans. Moreover, the ambiguity of the definition of what is a segment does not help in the solution of this issue (Hannibal and Patel 2013). Kinorhynchs have been largely considered pseudosegmented animals, demonstrated by the utilization of the term “zonite” instead of segment to differentiate their body plan from arthropods and annelids. However, they have serial cuticular structures as well as muscles and ganglia which are more similar of what the segmented annelids and arthropods have (Hannibal and Patel 2013). In this context the studies focused on the segmental organ systems of kinorhynchs, such as the nervous system and the musculature, are essential (Chapters VI, VII, Appendix II).

As a result of the studies carried out in this thesis we can confirm once more that there are no true segments in the head or neck region of kinorhynchs (Kristensen and Higgins 1991; Sørensen and Pardos 2008; Chapter V). This affirmation is supported by the presence of unsegmented and radially arranged longitudinal and circular musculature in the mouth cone, introvert and neck regions, which is completely different compared with the arrangement found in any of the trunk segments. Moreover, the general structure of a tripartite collar-shaped brain surrounding the intestine, present in kinorhynchs, is shared with remaining non-segmented cycloneuralians. Consequently, it is not very likely that the brain of kinorhynchs could be composed of the fusion of ganglia from “old” segments as in arthropods. However, developmental studies will be necessary in order to discover the origin and nature of segmental organ systems in Kinorhynchs.

The true delight is in the finding out rather than in the knowing

Isaac Asimov

5 Conclusions

5. Conclusions

The conclusions are arranged following the three main approaches proposed in the objectives.

Biogeography

- Samplings along twenty areas and more than a hundred stations along the Iberian Peninsula revealed a surprisingly high diversity of cyclorhagid kinorhynchs with 24 species grouped into 9 different genera, becoming one of the current best studied areas for the phylum and serving as a model for future investigations in other areas.

- No significant differences were found in taxon diversity among the fauna of the Atlantic vs. Mediterranean waters of the Iberian Peninsula. However, our samples are biased towards Atlantic localities and the results might not be very conclusive until more extensive sampling coverage is accomplished in Mediterranean localities.

- In the Iberian Peninsula 6 species show a clear wide distribution *(C. spinosus, D. gallaicus, E. dujardinii, E. hispanicus, Echinoderes* sp.1 and *Semnoderes armiger)* whereas apparently restricted distributions of some new undescribed species seems to be an artifact of incomplete sampling.

- Clear sediment preferences were detected by *Dracoderes gallaicus,* always associated with very fine mud, and species of *Antygomonas* normally associated with sandy or coarse sediments. Apparent depth preferences observed seem to be influenced by the sampling design (most samples in depths between 1-50 m).

- The apparently disjunct distribution of some taxa such as *Meristoderes, Dracoderes* and *Fissuroderes* around the world is most probably a reflection of isolated sampling. Consequently, it is expected to find these genera in intermediate areas.

- Sampling areas outside the Iberian Peninsula yielded high kinorhynch biodiversity, being Florida coasts the most diverse with 12 cyclorhagid species belonging to 7 different genera, followed by Naples with 7 species of three genera, and Panama with 5 species of three genera. However, these data might not

reflect the real diversity of the area yet.

• Current studies in Florida and Naples have yielded 6 and 4 new species respectively demonstrating that even well sampled areas still hold undiscovered diversity and that kinorhynch populations might change over time.

Taxonomy

• A total of 25 new cyclorhagid species and a new genus were found in the sampled areas, the new genus and 8 of the new species were described, while 17 species still await description.

• The redescription of *Dracoderes abei* with modern standards and including for the first time SEM data for the genus confirm the importance of the application of new microscopy techniques in taxonomy.

• SEM and LM are complementary techniques, essential for the accurate description of new kinorhynch taxa.

• The use of improved and more understandable diagrams in the study of kinorhynchs (polar diagrams of the introvert, diagrams of the trunk), facilitates the clarity and homogeneity of future descriptions as well as a straightforward comparison of characters among species and genera.

• The description of new morphological traits (e.g. accessory trichoscalids of *Fissuroderes*) and new standardized terminology (e.g. ij-line, free flap, fringe tips) improved and widen the set of reliable characters for future taxonomical and phylogenetical studies.

• Introvert morphology changes among species and genera and therefore could be a potential taxonomic tool to provide new clues about evolutionary pathways within Kinorhyncha. However, the amount of reliable information is still too limited and scattered across the taxa to make any conclusions.

• The key characters for the identification of cyclorhagid kinorhynch genera are the arrangement of cuticular plates of first and second trunk segments and the closing system type.

• Primary taxonomic characters at the species level are the number and arrangement of tubes, spines and glands, while the types and pattern of sensory spots are only useful secondary characters.

Morphology

• The use of CLSM combined with cytochemical and immunocytochemical techniques is currently the best approach to accurately describe the architecture of complex organ systems, such as muscular and nervous systems, of small creatures in whole mounts.

Regarding the myoanatomy:

• Within *Echinoderes* the external variability of cuticular structures is not reflected in the internal arrangement of the musculature that appears highly conserved among species.

• Myoanatomical differences among genera are concentrated in the anteriormost trunk segments, head, and neck due to the different closing systems, and in the musculature associated with the terminal spines, while the musculature of the trunk seems to be more conserved.

- Within Scalidophora, comparisons of the myoanatomy are only possible in the head region. Musculature is more similar between Kinorhyncha and Loricifera, than either group is to Priapulida and is clearly distinct from related vermiform cycloneuralian taxa near the base of Ecdysozoa.
- Kinorhynch myoanatomy may reflect an ancient transition from vermiform to segmented body plans during the early radiation of Ecdysozoa.

Regarding the nervous system:

- The adaptation of the standard protocols to the particular nature of kinorhynchs allowed to carry out successfully the first immunocytochemical study in the phylum.
- The combination of different markers (antibodies against serotonin and alpha acetylated tubulin with DNA staining) in three different genera and species allowed having a more complete view of the nervous system and its variability in the phylum.
- Cyclorhagid kinorhynchs show a modified orthogonal pattern in the arrangement of nerve fibers that could be remnants of an ancestral orthogonal arrangement.
- Mayor differences in the configuration of the nervous system of *Echinoderes, Antygomonas* and *Zelinkaderes* were detected in the neuropil, the ventral nerve cord and the distribution of the associated somata.
- The results on the nervous system architecture are relevant in a phylogenetic context and open new insights on the evolution of characters within Kinorhyncha, providing as well some hints to shed light to the origin and evolution of segmentation in ecdysozoans.

5. Conclusiones

Las conclusiones están organizadas siguiendo las tres líneas de estudio seguidas durante el desarrollo de la presente tesis doctoral.

Biogeografía

- Los muestreos realizados a lo largo de la Península Ibérica en 20 áreas y más de 100 estaciones de muestreo revelaron una biodiversidad muy elevada de kinorrincos ciclorrágidos con 24 especies pertenecientes a 9 géneros distintos. De esta forma la Península Ibérica se convierte en una de las áreas mejor estudiadas del mundo, sirviendo como ejemplo para futuros estudios en el filo.

- No se observaron diferencias significativas en la biodiversidad de kinorrincos entre las costas mediterráneas y atlánticas de la Península Ibérica. Sin embargo, nuestros muestreos están sesgados hacia la zona atlántica, por lo que los resultados no serán concluyentes hasta no recopilar más datos de la zona puramente mediterránea.

- En la península Ibérica 6 especies (*C. spinosus*, *D. gallaicus*, *E. dujardinii*, *E. hispanicus*, *Echinoderes* sp.1 and *Semnoderes armiger*) mostraron distribuciones claramente amplias, mientras que las distribuciones aparentemente restringidas, sobretodo de especies nuevas para la ciencia, podrían ser debidas a un muestreo incompleto.

- Claras preferencias de sedimento fueron detectadas por *Dracoderes gallaicus,* siempre asociado a fangos muy finos, y especies pertenecientes al género *Antygomonas,* asociadas a sedimentos arenosos gruesos. Las aparentes preferencias de profundidad podrían ser debidas al diseño de muestreo (siempre en zonas de profundidades comprendidas entre 1-50 m).

- Las distribuciones aisladas en distintas áreas del mundo para los géneros *Fissuroderes*, *Meristoderes* y *Dracoderes* son un reflejo de la falta de muestreos. Por ello se espera encontrar especies pertenecientes a éstos géneros en futuros estudios en áreas intermedias.

- Las áreas estudiadas fuera de la Península Ibérica revelaron también una elevada biodiversidad de kinorrincos ciclorrágidos siendo Florida la zona más diversa con 12 especies de 7 géneros, seguida de Nápoles 7 especies de 3 géneros y por último Panamá con 5 especies de 3 géneros. Sin embargo, estos resultados podrían no reflejar la diversidad real del área.

- Los actuales estudios desarrollados en Nápoles y Florida han descubierto 6 y 4 nuevas especies para la ciencia, demostrando que incluso áreas bien muestreadas todavía pueden contener diversidad sin descubrir, o bien que las poblaciones de kinorrincos pueden sufrir variaciones con el tiempo.

Taxonomía

- Un total de 25 especies de ciclorrágidos y un nuevo género fueron encontrados en las áreas muestreadas. El nuevo género junto con 8 de las especies fueron descritos, mientras que las 17 restantes todavía quedan por describir.

- La redescripción de *Dracoderes abei*, gracias a la utilización por primera vez de microscopía electrónica de barrido en el género, confirma la importancia de la aplicación de nuevas técnicas de microscopía en

taxonomía.

• La combinación de la microscopía electrónica de barrido (SEM) y la microscopía óptica (LM) es fundamental para una descripción completa y detallada de nuevos taxones de kinorrincos.

• La utilización de diagramas mejorados en el estudio de los kinorrincos (mejores diagramas polares), facilita la claridad y homogeneidad de futuras descripciones, así como una comparación directa de caracteres entre géneros y especies.

• La descripción de nuevos caracteres morfológicos (fila accesoria de tricoscálidas en *Fissuroderes*) y nueva terminología especializada (*ij-line, free flap, fringe tips*) mejora y amplía el conjunto de caracteres morfológicos para futuros análisis filogenéticos.

• La morfología del introverto varía entre especies y géneros de manera que puede ser una buena herramienta taxonómica, además de aportar nuevas pistas sobre patrones evolutivos en kinorrincos. Sin embargo, la cantidad de información viable es todavía limitada y dispersa en los distintos géneros y especies, por lo que aún no se pueden sacar conclusiones.

• La composición del primer y segundo segmento del tronco es fundamental en la identificación de los distintos géneros de los kinorrincos ciclorrágidos.

• El número y la posición de los túbulos, espinas y salidas glandulares es un carácter primario a nivel de especie, mientras que el tipo y posición de las áreas sensoriales se considera un carácter secundario útil.

Morfología

• El uso de la miscroscopía confocal láser (CLSM) combinada con técnicas de immunocitoquímica es actualmente la mejor manera de estudiar la complejidad de sistemas orgánicos, como la musculatura y el sistema nervioso, en animales microscópicos como los kinorrincos.

Sobre la anatomía muscular:

• En *Echinoderes* la gran variedad de los caracteres cuticulares mostrada externamente no se refleja internamente, donde la musculatura está bastante conservada entre especies.

• La mayoría de las diferencias en la anatomía muscular a nivel de género se concentran en los primeros segmentos del tronco, cabeza y cuello, debido a los diferentes sistemas de cierre mostrados. También hay diferencias en la musculatura asociada a las espinas terminales, mientras que la musculatura del tronco se mantiene bastante conservada entre géneros.

• Dentro de los escalidóforos las comparaciones en la anatomía muscular son sólo posibles en la zona de la cabeza. La musculatura es más similar entre loricíferos y kinorrincos que entre ambos y priapúlidos, y a su vez es claramente diferente del resto de los cicloneuralios.

• La anatomía muscular de los kinorrincos puede reflejar una transición ancestral desde planes corporales vermiformes hasta segmentados en la radiación inicial de los Artrópodos.

Sobre el sistema nervioso:

• La adaptación de los protocolos estándares a las particularidades de los kinorrincos permitieron llevar

a cabo de manera exitosa los primeros estudios inmunocitoquímicos en el filo.

• La combinación de distintos marcadores permitió obtener una visión más completa de la arquitectura del sistema nervioso en los distintos géneros estudiados y su variabilidad en el filo.

• El sistema nervioso de los kinorrincos ciclorrágidos muestra un patrón ortogonal modificado que podría haber derivado de un patrón ortogonal ancestral.

• Notables diferencias en la arquitectura del sistema nervioso de los tres géneros estudiados *(Antygomonas, Echinoderes* y *Zelinkaderes),* se concentran en la zona del neuropilo así como la cuerda nerviosa ventral y células asociadas.

• Los resultados obtenidos sobre la arquitectura del sistema nervioso en kinorrincos parecen relevantes a nivel filogenético, abriendo nuevas perspectivas sobre la evolución de caracteres en el filo, que a su vez podrían arrojar luz sobre la evolución de la segmentación en ecdisozoos.

6 References

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